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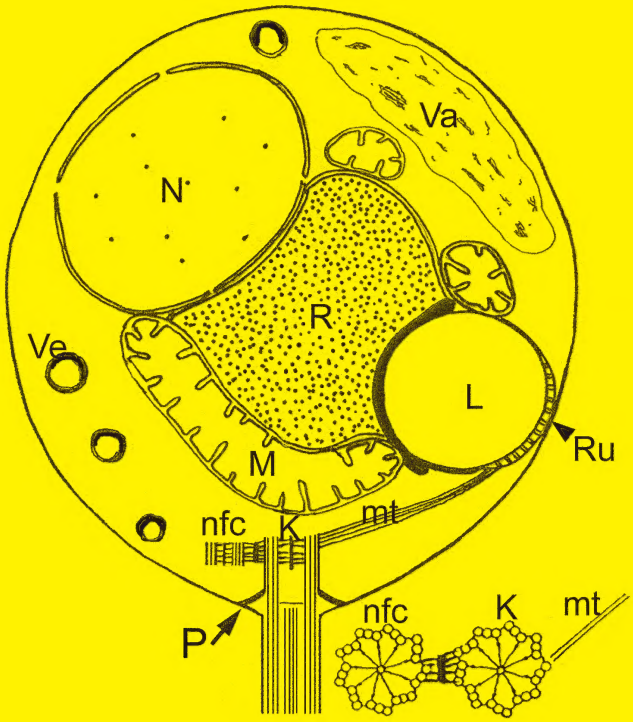

Homolaphlyctis polyrhiza Longcore, Letcher & T.Y. James, **gen. & sp. nov.**
(FIG. 4: zoospore ultrastructure, **p. 439**)
PETER LETCHER, ***artist***

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Errata from previous volumes

Volume 114

p. 429, line 4
for: Danilo. B
read: Danilo B.

Volume 117

p.20, lines 34–35
for: smooth under the light microscope, but with bacillate ornamentation under SEM (Figs.1 c–d, 2c).
read: smooth (Fig. 2b).

p.21, lines 17–19
for: yellowish to yellowish brown in KOH, smooth, subfusiform to ellipsoid, slightly thick-walled (up to 0.7 µm) (Fig. 2c).
read: yellowish to yellowish brown in KOH, subfusiform to ellipsoid, slightly thick-walled (up to 0.7 µm), smooth under the light microscope, but with bacillate ornamentation under SEM (Figs.1c–d, 2c).

p.27, line 20
for: **Albee-Scott S. 2007.**
read: Albee-Scott S. 2007.

p.27, line 42
for: Mycological Progress (Online First).
read: Mycological Progress 10: 389–398.

p.32, between ***Arthrobotrys latispora*** heading and Latin diagnosis:
add: MycoBank MB 560938 (assigned post publication)

p.368, line 10th from bottom
for: Mycobank 512114
read: MycoBank mb 560939 (assigned post publication)

Reviewers — volume one hundred eighteen

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this volume.

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From the Editor-in-Chief

Mycotaxon 118 — Welcome to the (almost) on schedule October–December 2011 volume! Granted, you are reading this in 2012, but we have great hopes that the hardcopies will be mailed in the first week of the first month of our new year. The year 2011 was the first where virtually all subscribers received their volumes entirely on-line. Where once we believed that online publication would be speedier, we now understand that our host service (ingentaconnect) needs almost a month to process a completed volume for downloading on their site, slightly over the time it took Sheridan Press to print and distribute our 2010 volumes. Nonetheless, 2012 will see each Mycotaxon volume published within the months written on the cover.

In the meantime, please enjoy Volume 118's 94 new taxa, names, typifications, and validations proposed by 172 authors (representing 30 countries) in 50 research papers reviewed by 83 experts. Color photos continue to illuminate, drawings are of such high quality that that selecting only one cover drawing remains challenging (we are happy to present our first cover chytrid in Mycotaxon 118), and the research published will be consulted for years to come.

Publication dates: nominal and actual — Nomenclatural priority is based on the actual date of effective publication. The International Code of Nomenclature for algae, fungi, and plants now permits effective publication in either printed or electronic format. We expected electronic publication to shorten (considerably) the time between a pdf's leaving the editorial desk and publication. However, because we still send hard copies to libraries well before the volume becomes visible online, the actual publication date will be based on that earlier distribution date.

A short while ago we discovered that ingentaconnect was citing the first day of the first month of a nominal quarter as 'publication date.' Thus, the delayed 2011 January–March volume, actually published on May 9, is cited on the download page as published on January 1, 2011. Plans are underway to correct the confusion, but until then, the date of effective publication is most reliably found on our "volume listings: publication history" publications webpage via www.mycotaxon.com. Remember that Mycotaxon always cites the actual publication date of the previous volume in the cover section of the next volume (see p. vii, this section).

Mycotaxon in 2012 and beyond — I wish to thank our authors and expert reviewers, the Editorial Advisory Board, and most especially the Mycotaxon staff (*Nomenclature Editor* Shaun Pennycook, *Book Review Editor* Else Vellinga, *Webmaster* Noni Korf, *Business Manager* Hannes Maddens, and *Treasurer & Mentor* Dick Korf) for helping us continue to offer Mycotaxon, the most highly respected International Journal of Fungal Taxonomy & Nomenclature.

With warm best wishes for the New Year,
Lorelei Norvell, Mycotaxon *Editor-in-Chief*
December 30, 2011

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New records of *Geastrum* from Japanese sand dunes

Taiga Kasuya[1*], Kentaro Hosaka[2], Haruo Sakamoto[3], Akitomo Uchida[4], Tamotsu Hoshino[5, 6] & Makoto Kakishima[1]

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Abstract— Basidiomata of three earthstars —*Geastrum campestre, G. corollinum, G. hungaricum*— were collected from sand dunes of the Japanese coasts. Those arenicolous fungi are newly recorded for Japanese mycobiota. Descriptions, comments and illustrations of basidiomata of the three fungi are provided.

Key words— biogeography, coastal environment, gasteromycetes, *Geastrales*, taxonomy

Introduction

The genus *Geastrum* Pers. belongs to *Geastrales, Phallomycetidae* (Hosaka et al. 2006) and includes currently about 50 taxa (Kirk et al. 2008). Most species of the genus are known as saprobes and are distributed throughout all continents except Antarctica. Several authors have systematically revised *Geastrum* (Staněk 1958, Ponce de Leon 1968, Dörfelt & Müller-Uri 1984, Dörfelt & Heklau 1987, Sunhede 1989). Several species have been recorded from multiple continents, although their taxonomic identities are doubtful because there are only a few molecular phylogenetic studies. Our preliminary morphological and phylogeographical analyses of the globally distributed "*G. triplex* Jungh." indicate that the name represents an aggregation of multiple species (Kasuya et al. 2011a). This suggests the possible existence of many cryptic species in the genus.

Although 18 *Geastrum* species have hitherto been recognized from Japan (Imai 1936, Kawamura 1954, Ito 1959, Yoshimi & Hongo 1989, Sakamoto & Kasuya 2008, Kasuya et al. 2009, 2011b), comprehensive taxonomical and biogeographical studies of the genus have not yet been conducted. Therefore, to clarify the geographical distribution, morphological variations, and phylogenetic positions of Japanese *Geastrum*, we conducted morphological and molecular phylogenetic analysis of the genus based on the materials from multiple localities including Japan and other continents (Kasuya et al. 2011a).

Several *Geastrum* species favor semiarid to arid environments, e.g., well-drained sandy soils of coasts, deserts, and inland steppes. During our recent floristic and taxonomic investigations of Japanese *Geastrum* (Sakamoto & Kasuya 2008, Kasuya et al. 2009, 2011b), we recorded *G. kotlabae* V.J. Staněk, *G. minimum* Schwein., and *G. quadrifidum* Pers. from coastal sand dunes of Hokkaido and Honshu.

Some noteworthy *Geastrum* collections obtained by further fieldwork conducted in sand dunes along the Japanese coasts include *G. campestre*, *G. corollinum,* and *G. hungaricum,* which represent new distributional records for Japan. We describe the Japanese collections of these three species with the aid of illustrations showing morphological characters. We also compare them with related taxa and discuss the biogeographical and ecological features of the species.

Materials & methods

Fieldwork was carried out from September 2003 to October 2010 at three sites in coastal sand dunes of Hokkaido and Honshu, Japan: (1) Minehama, Shari, Hokkaido (43°55′34″N, 144°46′18″E); (2) Hitachi Kaihin Park, Hitachinaka, Ibaraki (36°23′54″N, 140°36′24″E); and (3) Fukude, Iwata, Shizuoka (34°39′51″N, 137°53′22″E). These sites well preserve coastal vegetation dominated by poaceous and cyperaceous plants. For morphological comparisons, we examined one additional *G. corollinum* specimen collected from an inland area of Honshu.

The examined specimens are deposited in the mycological herbarium of the National Museum of Nature and Science, Tsukuba, Ibaraki, Japan (TNS). Macroscopic characters were described from dried and fresh material. For light microscopic observations, freehand sections of the specimens were mounted in water, 3% (w/v) KOH, and 30% ethanol solution on glass slides. Forty randomly selected basidiospores were measured under a light microscope at 1000× magnification. Length measurements excluded the apiculus; average dimensions ± standard deviations are shown in brackets. The surface features of basidiospores were also observed by scanning electron microscopy (SEM). For SEM, a small portion of the gleba was dusted onto double-sided adhesive tape on a specimen holder, coated with platinum-palladium using an E-1030 Ion Sputter Coater (Hitachi, Tokyo, Japan), and examined with a S-4200 SEM (Hitachi, Tokyo, Japan) operating at 20 kV.

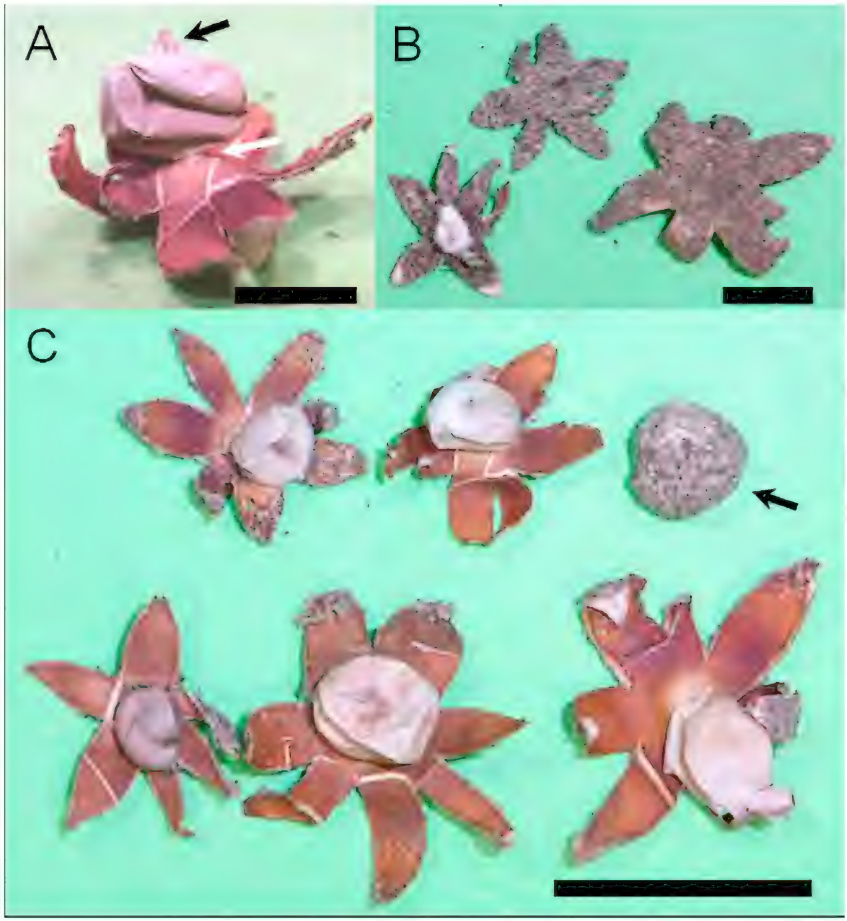

FIGURE 1. *Geastrum campestre*: macrocharacters (TNS–F–38710). A: Detailed structure of expanded basidiomata. Note plicate peristome (black arrow) and short stalk (white arrow). B: The mycelial layer of expanded basidiomata. C: Expanded and an unexpanded (arrow) basidiomata. Scale bars = 20 mm.

Taxonomy

Geastrum campestre Morgan, Amer. Nat. 21: 1027, 1887, as "*Geaster campester*". FIGS. 1–2

JAPANESE NAME: Hamabe-no-hida-tsuchigaki (coastal dune's plicate earthstar, newly named here).

UNEXPANDED BASIDIOMATA hypogeous to subhypogeous, depressed globose, globose to subglobose when young, ca. 5–12 mm diam., surface encrusted

with sand, whitish to slightly cineraceous. Expanded basidiomata 8–32 mm across when dried, 15–37 mm across when wetted, exoperidium splitting into 6–8 rays, weakly to strongly hygroscopic, rays arched with straight to slightly recurved tips in fresh state or when wetted, then partly or entirely covering the endoperidial body when old or dried. Mycelial layer thin, whitish, encrusted with sand and plant debris, persistent, attached to the fibrous layer for a long time and without forming basal remnants attached to the basidiomata. Fibrous layer outer side almost completely covered with the mycelial layer, whitish to grey. Pseudoparenchymatous layer persistent, pale beige in fresh state, later becoming beige, reddish brown to dark brown. Endoperidial body stalked, depressed globose, globose to subglobose, 3–11 mm diam., with or without apophysis. Endoperidium pale brown, greyish brown to dark brown, finely pruinate or almost smooth when old, but usually distinctly pruinose or tomentose with whitish to beige crystalline material in fresh basidiomata, with an indistinct circular area surrounding the peristome. Peristome strongly plicate with 13–22 folds, almost concolorous or somewhat darker than endoperidium, broadly conical to mammiform, distinctly delimited, 1–2.5 mm long. Stalk short but distinct, 0.5–1 mm long. Columella globose, ovoid to club-shaped in mature state, persistent, distinct. Mature gleba olivaceous brown to brown.

Mycelial layer consisting of dimorphic hyphae: (I) 2–5 μm thick, hyaline, thick-walled, rarely branched; (II) 1.5–3.5 μm thick, hyaline, thin-walled, with clamp-connections, rarely branched. Fibrous layer consisting of 2.5–6.5 μm thick, hyaline, thick-walled hyphae. Pseudoparenchymatous layer consisting of hyaline, yellowish brown to pale brown, thick-walled, almost bladder-like but variously shaped cells. Endoperidium consisting of dimorphic hyphae: (I) 3.5–4.5 μm thick, yellowish brown, brown to dark brown, thick-walled, rarely dichotomously branched; (II) 2–4.5 μm thick, hyaline to pale yellowish brown, thin-walled, branched. Hyphae of the columella 2–6.5 μm thick, hyaline, pale yellowish brown to pale brown, thick-walled, rarely dichotomously branched. Hyphae of the capillitium hyaline to yellowish brown, thick-walled, 2–8 μm thick, tapered gradually towards subacute tips, dichotomously branched, surface usually encrusted with numerous amorphous remnants or crystalline materials but sometimes almost smooth. Basidia not seen. Basidiospores globose, densely verrucose, thick-walled, yellowish brown, 4.1–[4.9±0.4]–5.7 μm diam. excluding ornaments, 5–[5.5±0.3]–6.2 μm diam. including ornaments, verrucae conical to columnar-like, ≤ 0.8 μm high, with flat, rounded to subacute apexes, basal apiculus prominent.

Habitat and distribution: Uncommon in Japan, where it is gregarious on sand in coastal dunes in a warm-temperate zone near *Carex kobomugi*, *C. pumila,* and *Elymus mollis*. Several fresh basidiomata were collected in

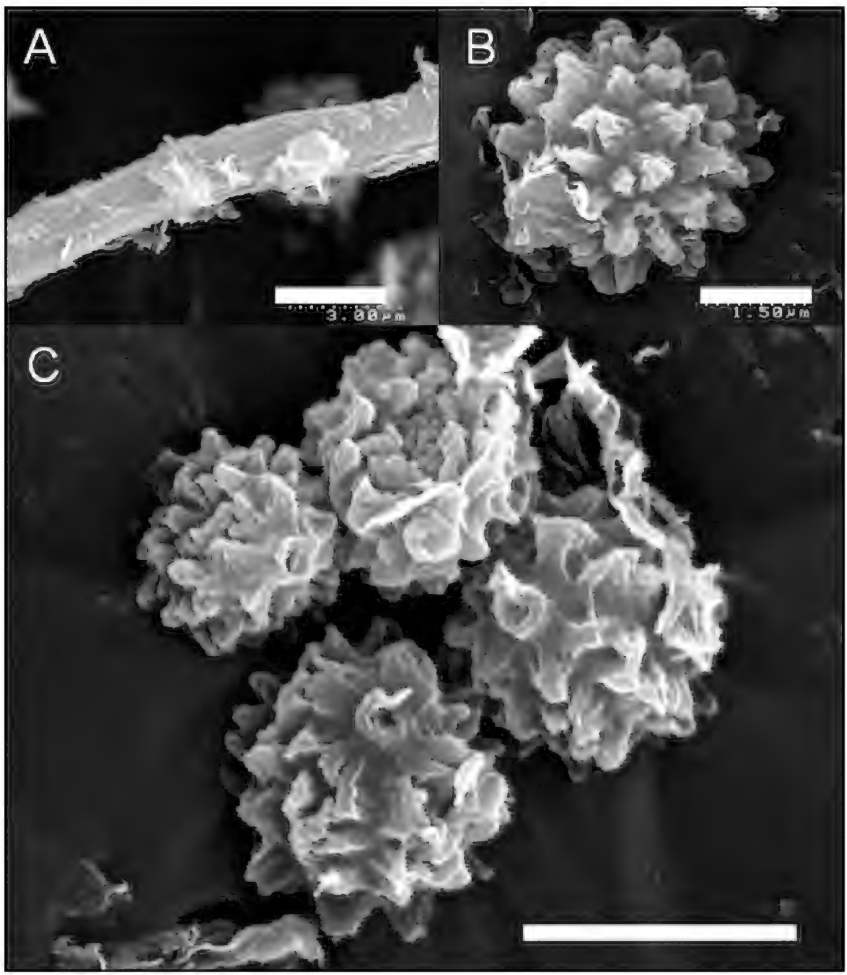

FIGURE 2. *Geastrum campestre*: SEM micrographs (TNS–F–38710). A: Hypha of the capillitium encrusted with amorphous remnants and crystalline materials. B: A basidiospore covered with dense verrucae. C: Basidiospores with prominent apiculus. Scale bars: A = 3 μm; B = 1.5μm; C = 2 μm.

September. Known from Japan (Ibaraki, new record), Europe (Sunhede 1989), South Africa (Smith 1935), North America (Long & Stouffer 1948), Central America (Esqueda et al. 2003), Hawaii (Gilbertson et al. 2001), and Australia (Cunningham 1944).

SPECIMENS EXAMINED: **JAPAN. IBARAKI PREFECTURE:** Hitachinaka-shi, Nagasuna, Hitachi Kaihin Park: September 26, 2004, H. Sakamoto, TNS-F-38710.

COMMENTS: *Geastrum campestre* is well characterized by hygroscopic exoperidial rays, a mycelial layer encrusted with sand or debris, a stalked endoperidial body, and a distinctly delimited, plicate peristome. The morphology of the Japanese specimens agrees well with previous descriptions of *G. campestre* (Cunningham 1944, Long & Stouffer 1948, Sunhede 1989). However, the expanded Japanese basidiomata are usually smaller (≤ 37 mm wide when wet) than European specimens (≤ 65 mm wide, Sunhede 1989).

Geastrum kotlabae and *G. pouzarii* V.J. Staněk are morphologically and ecologically very similar to *G. campestre*. All three species have hygroscopic exoperidial rays and plicate peristomes and share similar habitats such as well-drained, sandy soil. However, the endoperidial body of *G. kotlabae* is completely sessile (Sunhede 1989, Sakamoto & Kasuya 2008). Basidiospores of *G. pouzarii* are larger (5.5–7 µm diam. including ornaments, Sunhede 1989) than those of *G. campestre*. Moreover, the mycelial layer of both *G. kotlabae* and *G. pouzarii* easily peels off at basidiome expansion (Sunhede 1989, Esqueda et al. 2003) whereas that of *G. campestre* remains attached to the fibrous layer for a long time. *Geastrum berkeleyi* Massee, which also produces stalked endoperidial bodies and plicate peristomes, clearly differs from *G. campestre* by its non-hygroscopic, much larger basidiomata (Kasuya et al. 2009).

Japanese material of *G. campestre* has been collected from sand dunes along the Pacific Ocean coast in the warm-temperate area of Eastern Honshu. The type specimen of *G. campestre* was collected from Nebraska, U.S.A., where it grows on dry open grasslands or on litter under conifers or deciduous trees (Long & Stouffer 1948). European and Australian specimens have been obtained from well-drained, sandy soil near coasts (Cunningham 1944, Sunhede 1989), and the species has been recorded from tropical deciduous forests in Mexico (Esqueda et al. 2003) and dry mountain forests in Hawaii (Gilbertson et al. 2001). These facts suggest that *G. campestre* prefers dry, well-drained open land but can grow in a variety of environments.

Geastrum campestre is known from all continents except Antarctica and its habitats are diverse. As several morphological variations have been reported (Sunhede 1989), *G. campestre* as currently defined may be a species complex.

Geastrum corollinum (Batsch) Hollós, Gasterom. Ung.: 65, 1904, as "*Geaster corollinus*". FIGS. 3–4

JAPANESE NAME: Chijire-tsuchigaki (Curled earthstar, newly named here).

UNEXPANDED BASIDIOMATA usually epigeous, rarely subhypogeous, subglobose, ovoid to onion-shaped with an umbo, 6.5–13 mm wide, 14–17.5 mm high including apical umbo, surface almost smooth to minutely wrinkled and not encrusted with sand or plant debris, ochraceous to pale brown. EXPANDED BASIDIOMATA 10–24 mm across when dried, 16–28 mm across when wetted,

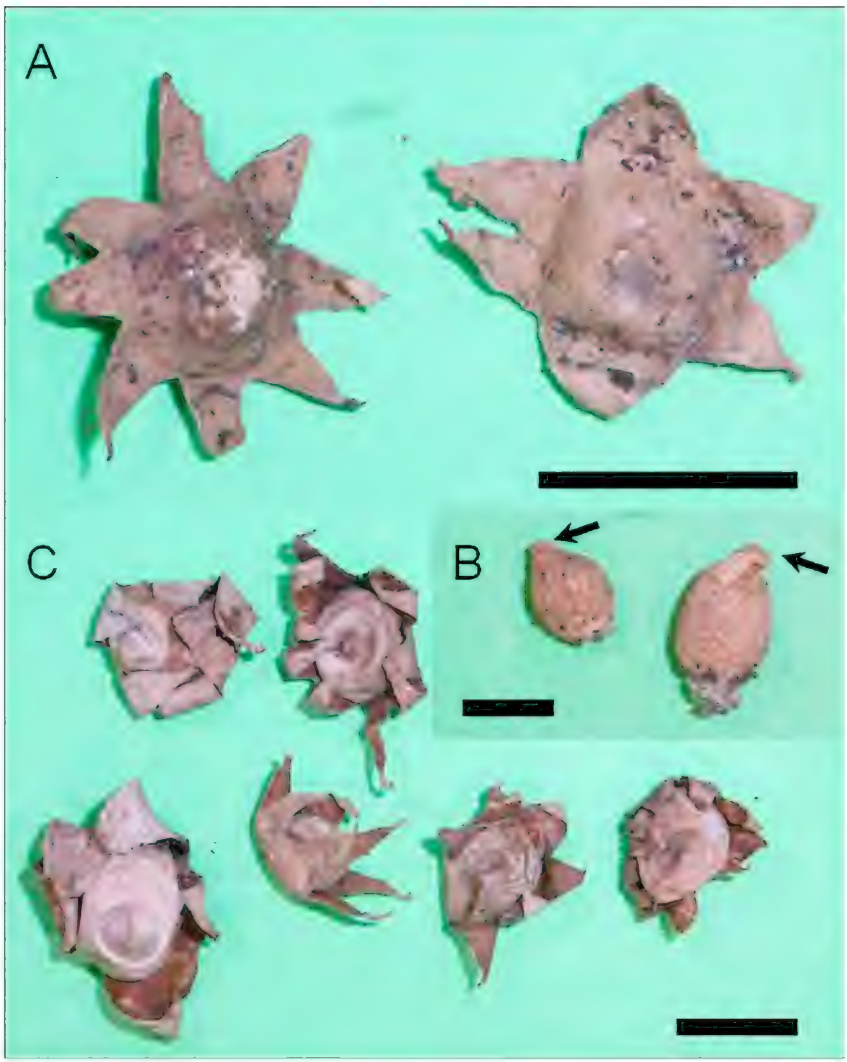

FIGURE 3. *Geastrum corollinum*: macrocharacters (TNS–F–38711). A: Two expanded basidiomata with distinctly delimited, fibrillose peristomes. B: Two unexpanded basidiomata with almost smooth surfaces. Note apical umbos (arrows). C: Expanded basidiomata showing hygroscopic exoperidial rays. Scale bars: A = 10 mm; B–C = 15 mm.

exoperidium splitting into 5–10 rays, hygroscopic, partly or entirely covering the endoperidial body and thus curled when dried, tips of the rays sometimes become recurved downwards in fresh state. MYCELIAL LAYER thin, yellowish

white, pale ochraceous to pale brown, smooth, without sand or plant debris, visible when unexpanded, but soon disappeared when basidiome expand. Fibrous layer firm, persistently attached to the pseudoparenchymatous layer, outer side ochraceous to pale brown. Pseudoparenchymatous layer persistent, thin (>0.5 mm thick), pale beige in fresh state, later becoming ochraceous, reddish brown to dark brown, coriaceous when wetted or young, very hard when dried or old. Endoperidial body sessile, depressed globose, globose to subglobose, 6–11.5 mm diam., without apophysis. Endoperidium pale brown to greyish brown, almost smooth when old, but usually pruinate with whitish crystalline material in fresh or young basidiomata, with a circular area surrounding the peristome. Peristome surface fibrillose, almost concolorous or somewhat darker than endoperidium, broadly conical to mammiform, distinctly delimited, 1–3.5 mm long. Columella cylindrical to club-shaped, slender, sometimes indistinct. Mature gleba olivaceous brown to brown.

Mycelial layer consisting of dimorphic hyphae: (I) 2–4.5 μm thick, hyaline, thick-walled, rarely branched; (II) 2–3.5 μm thick, hyaline, thin-walled, with clamp-connections, branched. Fibrous layer consisting of 3.5–7 μm thick, hyaline to pale yellowish brown, thick-walled hyphae. Pseudoparenchymatous layer consisting of thick-walled, hyaline, yellowish brown to reddish brown, almost bladder-like but variously shaped cells. Endoperidium consisting of dimorphic hyphae: (I) 3.5–6 μm thick, hyaline to pale yellowish brown, thick-walled, rarely dichotomously branched; (II) 1.5–4.5 μm thick, hyaline to pale yellowish brown, thin-walled, branched. Hyphae of the columella 2–7 μm thick, thick-walled, surface smooth, hyaline to pale yellowish brown. Hyphae of the capillitium 2–6.5 μm thick, thick-walled, pale yellowish brown to yellowish brown, curved, tapered gradually towards subacute tips, rarely dichotomously branched, surface almost smooth or sometimes encrusted with amorphous remnants and crystalline materials. Basidia not seen. Basidiospores globose, densely verrucose, thick-walled, yellowish brown to olivaceous brown, 3.5–[4±0.3]–4.5 μm diam. excluding ornaments, 4–[4.7±0.4]–5.5 μm diam. including ornaments, verrucae conical to columnar-like, ≤ 0.7 μm high, with flat, rounded to subacute apexes, basal apiculus prominent.

Habitat and distribution: Uncommon in Japan, solitary to gregarious on sand near *Carex kobomugi* and *C. pumila* in coastal dunes or on the ground in broad-leaved forests in a warm-temperate area. Fresh and old basidiomata were collected in July and December. Known from Japan (Shizuoka and Osaka, new record), China (Zhou et al. 2007), Europe (Hollós 1904, Sunhede 1989), South Africa (Bottomley 1948; Coetzee et al. 1997), North America (Coker & Couch 1928), Central America (Esqueda et al. 2003) and Hawaii (Gilbertson et al. 2001).

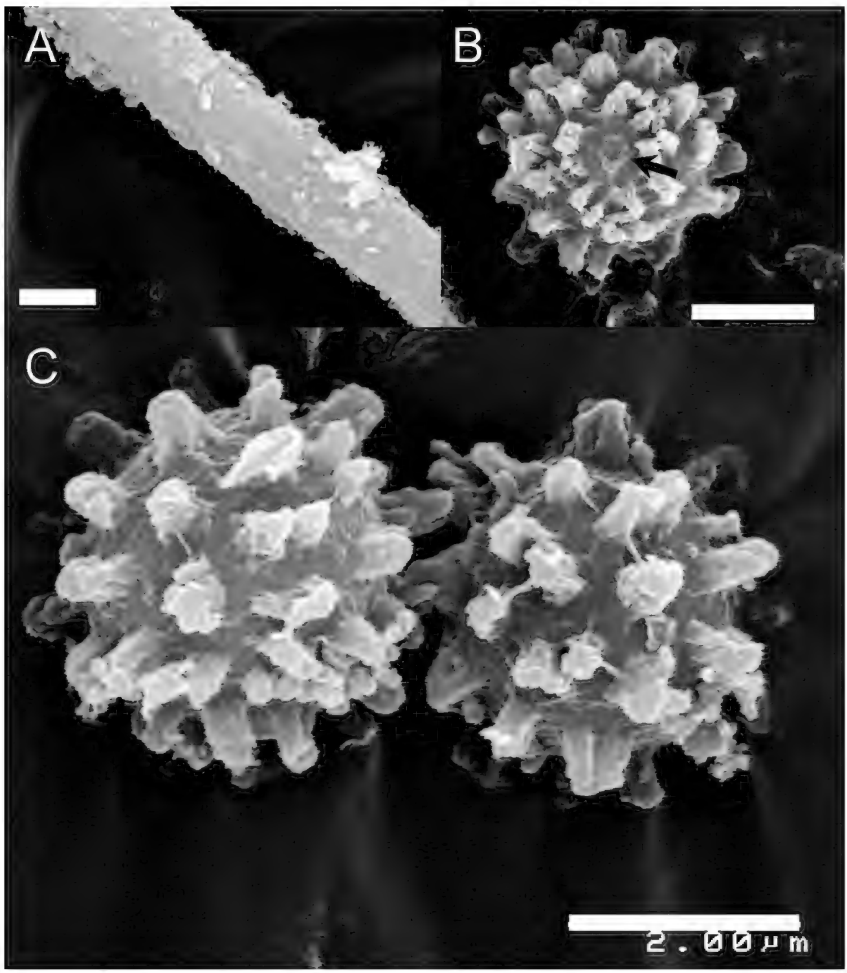

FIGURE 4. *Geastrum corollinum*: SEM micrographs (TNS–F–38711). A: Hypha of the capillitium encrusted with amorphous remnants and crystalline materials. B: A basidiospore with apiculus (arrow). C: Basidiospores with dense verrucae. Scale bars = 2 µm.

SPECIMENS EXAMINED: **JAPAN. SHIZUOKA PREFECTURE:** Iwata-shi, Fukude: December 3, 2003, I. Asai, TNS-F-38711. **OSAKA PREFECTURE:** Katano-shi, Kaigake-no-michi: July 31, 2011, Y. Kotera, TNS-F-550009.

COMMENTS: *Geastrum corollinum* is diagnosed by hygroscopic exoperidial rays, a smooth mycelial layer not encrusted with sand or debris, and a distinctly delimited, fibrillose peristome. The morphology of the Japanese specimens

agrees well with previous descriptions of *G. corollinum* (Hollós 1904, Dissing & Lange 1962, Sunhede 1989). However, the pseudoparenchymatous layer of the exoperidium of the Japanese material is thinner (≤ 0.5 mm thick) than that of European material (0.5–1.5 mm thick, Sunhede 1989).

Geastrum floriforme Vittad. and *G. hungaricum* are morphologically similar to *G. corollinum*. However, *G. floriforme* basidiomata are hypogeous when unexpanded and have indistinctly delimited peristomes and the mycelial layer is encrusted with sand or plant debris (Sunhede 1989). For comparison with *G. hungaricum*, see description and comments below. *Geastrum arenarium* Lloyd, which resembles *G. corollinum* macroscopically, differs in its mycelial layer strongly encrusted with sand or plant debris (Sunhede 1989) and smaller basidiospores of *G. arenarium* (3.5–4.5 μm diam. including ornaments, Sunhede 1986).

Japanese specimens of *G. corollinum* have been collected from coastal sand dunes along the Pacific Ocean and under broad-leaved forests of warm-temperate areas of Central Honshu. In Europe, the type locality of *G. corollinum*, it has been obtained from various habitats, under deciduous trees (Dissing & Lange 1962, Sunhede 1989), under *Juniperus communis* (Sunhede 1989), and in sandy grasslands (Dörfelt et al. 1979) and coastal dunes (Eyndhoven 1937). Chinese specimens have been collected from grasslands, grazed fields and under *Cryptomeria japonica* or several salicaceous trees (Zhou et al. 2007). *Geastrum corollinum* has also been recorded from tropical deciduous forests in Mexico (Esqueda et al. 2003) and dry, coastal to montane forests in Hawaii (Gilbertson et al. 2001). Ecologically, these facts suggest that the present fungus adapts to varied environments. In Japan, further fieldwork is needed to clarify whether the distribution of *G. corollinum* is limited to coastal sand dunes or not.

Geastrum corollinum has been recorded from all continents except Antarctica. Given the variable morphological characters (Dissing & Lange 1962, Sunhede 1989) and diverse habitats, *G. corollinum* probably comprises a species complex.

Geastrum hungaricum Hollós, Math. Természettud. Értes. 19(5): 506, 1901, as "*Geaster hungaricus*". FIGS. 5–6

JAPANESE NAME: Arechi-no-himetsuchiguri (dry wastelands earthstar, newly named here).

UNEXPANDED BASIDIOMATA hypogeous to subhypogeous, depressed globose, subglobose to ovoid, ca. 3–10 mm diam., surface encrusted with sand and plant debris, whitish to slightly cineraceous. EXPANDED BASIDIOMATA 5–12 mm across when dried, 13–17 mm across when wetted, exoperidium splitting into 6–11 rays, strongly hygroscopic, partly or entirely covering the endoperidial body when dried, tips of the rays sometimes become curved downwards in

FIGURE 5. *Geastrum hungaricum*: macrocharacters. A: An expanded basidioma (TNS–F–38713) with distinctly delimited, fibrillose peristome. B: Expanded basidiomata (TNS–F–38712) showing strongly hygroscopic exoperidial rays. Note basal remnants of the mycelial layer attached to basidiomata with sand and plant debris (arrows). C: An expanded basidioma (TNS–F–38713) in the natural habitat. Scale bars: A, C = 5 mm; B = 10 mm.

fresh state. MYCELIAL LAYER thin, whitish, with sand and plant debris, easily peeling off and usually forming basal remnants attached to the basidiomata. FIBROUS LAYER firmly, persistently attached to the pseudoparenchymatous layer, outer side white. PSEUDOPARENCHYMATOUS LAYER persistent, cream to pale beige in fresh state, later becoming pale brown, reddish brown to

dark brown. Endoperidial body sessile, globose, subglobose to ovoid, 2–9 mm diam., without apophysis. Endoperidium pale brown to greyish brown, almost smooth when old, but usually pruinate with whitish to beige crystalline material in fresh basidiomata, with a circular area surrounding the peristome. Peristome surface fibrillose, almost concolorous or somewhat darker than endoperidium, discoid to broadly conical, distinctly delimited, 0.5–1.5 mm long. Columella cylindrical, slender, sometimes indistinct. Mature gleba olivaceous brown to brown.

Mycelial layer consisting of dimorphic hyphae: (I) 2–5.5 μm thick, hyaline, thick-walled, rarely branched; (II) 2–5 μm thick, hyaline, thin-walled, rarely with clamp-connections, sparsely branched. Fibrous layer consisting of 3–7.5 μm thick, hyaline to pale yellowish brown, thick-walled hyphae. Pseudoparenchymatous layer consisting of thick-walled, hyaline to pale brown, almost bladder-like but variously shaped cells. Endoperidium consisting of 2.5–5 μm thick, hyaline, pale yellowish brown to pale brown, thick-walled hyphae encrusted with amorphous crystalline materials. Hyphae of the columella 1–6 μm thick, thick-walled, surface smooth, pale yellowish brown. Hyphae of the capillitium 1–8 μm thick, thick-walled, pale yellowish brown to yellowish brown, tapered gradually towards subacute tips, sometimes dichotomously branched, surface almost smooth or encrusted with some amorphous remnants and crystalline materials. Basidia not seen. Basidiospores globose, warty, thick-walled, olivaceous brown to dark brown, 4.3–[4.9±0.3]–5.3 μm diam. excluding ornaments, 4.8–[5.4±0.3]–5.8 μm diam. including ornaments, warts radiating from the base of the apiculus and connecting to adjacent verrucae thus showing wing-like, ≤ 0.5 μm high, with rounded to subacute apexes, basal apiculus prominent.

Habitat and distribution: Uncommon in Japan, gregarious or solitary on sand among mosses near *Elymus mollis* in coastal dunes in a cool-temperate area. One fresh basidioma was collected in September; several overwintered, old specimens were found in May. Known from Japan (Hokkaido, new record), Mongolia (Dörfelt & Otto 1985), Russian Karachay-Cherkessia (Hollós 1904), Hungary (Hollós 1904), Czech Republic (Staněk 1958), and Germany (Dörfelt et al. 1979).

Specimens examined: **JAPAN. Hokkaido:** Shari-gun, Shari-cho, Minehama: May 29, 2009, T. Hoshino and A. Uchida, TNS-F-38712; September 27, 2010, A. Uchida, TNS-F-38713.

Comments: *Geastrum hungaricum,* one of the smallest *Geastrum* species of the genus, is well characterized by strongly hygroscopic exoperidial rays, an endoperidium with a pruinose surface with whitish to beige crystalline material, and a distinctly delimited, fibrillose peristome. The morphology of the Japanese specimens agrees well with previous descriptions of *G. hungaricum* (Hollós

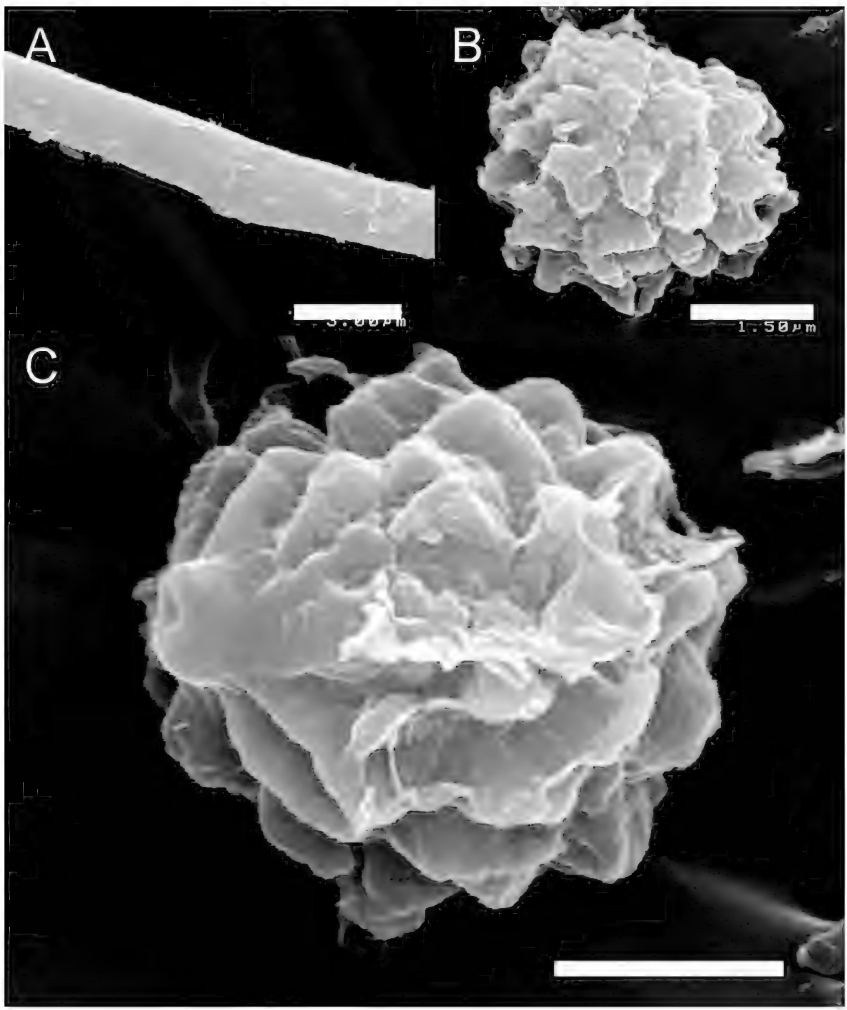

FIGURE 6. *Geastrum hungaricum*: SEM micrographs. A: Hypha of the capillitium sparsely encrusted with amorphous remnants and crystalline materials (TNS–F–38712). B: A basidiospore covered with dense verrucae (TNS–F–38712). C: A basidiospore with prominent apiculus (TNS–F–38713). Note wing-like verrucae radiating from the base of the apiculus. Scale bars: A = 3 µm; B–C = 1.5 µm.

1904, Staněk 1958, Sunhede 1989), except that the basidiospores are slightly narrower than in European materials (5–6 µm diam. including ornaments, Sunhede 1989).

Two other small-sized earthstars, *G. corollinum* and *G. floriforme,* resemble *G. hungaricum*. However, unexpanded basidiomata of *G. corollinum* are epigeous, onion-shaped with an umbo, with brownish surfaces, and not encrusted with sand or plant debris (Sunhede 1989; see also our description of *G. corollinum*, above) and the expanded basidiomata are larger (≤ 23 mm diam., Sunhede 1989). *Geastrum floriforme* clearly differs from *G. hungaricum* by its indistinctly delimited peristome, smooth endoperidial surfaces, and larger basidiospores (≤ 7 μm diam., Sunhede 1989).

Japanese material of *G. hungaricum* was collected from a coastal sand dune along the Sea of Okhotsk in the cool-temperate area of eastern Hokkaido, while the type was collected from Hungary. In Europe it has been collected from sandy, rocky or grazed ground (Hollós 1904, Staněk 1958, Sunhede 1989), and Russian or Mongolian specimens were also found in semiarid areas (Hollós 1904, Dörfelt & Otto 1985), suggesting that *G. hungaricum* habitats are limited to dry, sandy, stony or grazed ground in northern cool-temperate regions.

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Discrimination of *Gigaspora* species by PCR specific primers and phylogenetic analysis

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Abstract — Species of arbuscular mycorrhizal fungi (AMF) are usually identified by the morphological characteristics of their spores. However, considering the difficulties in diagnosing their taxa, the construction of species-specific primers has been proposed as an identification alternative. In this paper the problem of distinguishing different *Gigaspora* species with slight morphological differences was solved using species-specific primers and SSU and LSU rDNA sequence analyses of 18 AM fungal isolates comprising seven species. Neighbor joining, maximum parsimony, and maximum likelihood analyses were performed to evaluate the phylogenetic affiliation of the isolates, and a new reverse PCR primer (ALB1) specific for *Gigaspora albida* was designed and tested with 11 *Gigaspora* isolates (four species). The results confirmed misidentification of '*G. albida*' FL 927 and '*G. margarita*' BR 444 and supported referring FL 927 to *G. rosea* and BR 444 to *G. albida*.

Key words — phylogeny, ribosomal sequences, *Glomeromycota*

Introduction

There are approximately 220 species of arbuscular mycorrhizal fungi (AMF) (Stockinger et al. 2010). Morphological differences in spore structure are usually used to distinguish between individual species, but this requires a great deal of experience (Morton 1993, Bentivenga & Morton 1994). More practical methods are needed, so that AMF can be identified directly not only in the rhizosphere, but also after host root colonization. DNA analytical methods involving electrophoretic profiles (Wyss & Bonfante 1993), sequence comparisons (Daniell et al. 2001, Husband et al. 2002, Mummey & Rillig 2007, Stukenbrock & Rosendahl 2005), PCR-DGGE (Souza et al. 2004), species-

specific primers (Gamper & Leuchtmann 2007, Geue & Hock 2004, Lanfranco et al. 1995, 1999, 2001, Millner et al. 1998, 2001a, 2001b, Redecker 2000), and DNA barcodes (Stockinger et al. 2010) are being developed that would allow identification of individual AMF species in an ecological context. For example, species-specific PCR primers could be used to discriminate morphologically similar species or even to identify species within colonized roots. However, to date only a small number of species-specific probes has been developed.

After Oehl et al. (2008) divided the *Gigasporaceae* into four families and six genera, Morton & Msiska (2010) proposed dividing *Gigasporaceae* into just three genera —*Gigaspora*, *Racocetra, Scutellospora. Gigaspora* has five species (Bentivenga & Morton 1995) in which the morphological variation is low (Bago et al. 1998, Souza et al. 2004, Lanfranco et al. 2001), and only diameter, color and spore wall thickness of glomerospores have been used for species identification (Bentivenga & Morton 1995). Species-specific primers designed for *G. margarita* (Lanfranco et al. 1999) and *G. rosea* (Lanfranco et al. 2001) have shown to be effective in solving such morphological conflicts. In this study we show how a species-specific PCR primer constructed for *G. albida* helps solve problems associated with discriminating *Gigaspora* species and evaluate *Gigaspora* phylogenetically through SSU and LSU rDNA analyses.

Materials & methods

AM fungi

Eleven reference isolates (Table 1) representing *Gigaspora* propagated in pot cultures were selected.

Intracellular bacteria

Presence of intracellular bacteria was assessed with the Live/Dead *Bac*-Light bacterial viability kit (Molecular Probes, Inc., Eugene, OR, USA) following Bianciotto et al. (1996).

DNA extraction

Ten to 50 spores were washed in distilled water, sonicated 3–4 times, crushed in 50–100 µl of 1x REDTaq PCR Reaction Buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.1 mM $MgCl_2$ and 0.01% gelatin) (Sigma-Aldrich, Milan, Italy), and centrifuged at 1000 RPM for 2 min. The supernatant containing the DNA was incubated at 95°C for 13 min. After extraction, the DNA was stored at –20°C.

Design of PCR primers, amplification and sequencing

A new species-specific reverse PCR primer ALB1 (5'-CCCAACTAAAAATACTTCAGTC-3') was designed for *G. albida* based on the GenBank ITS sequence AJ239118 and combined with GiITS1 (Lanfranco et al. 1999) to create a 385 bp fragment. For *G. margarita* and *G. rosea,* combinations of primers GiITS1+GiITS2 (Lanfranco et al. 1999) and GiITS1-GiR3 (Lanfranco et al. 2001) were used, respectively.

Table 1. *Glomeromycota* isolates used in PCR amplification tests with species-specific primers and phylogenetic analysis.

Species[a]	Isolate	Origin	Source[b]
Gigaspora albida N.C. Schenck & G.S. Sm.	BR 601	Brazil	INVAM
G. albida	FL 927	USA	UFPE
G.decipiens I.R. Hall & L.K. Abbott	BEG 45	Australia	BEG
G. gigantea (T.H. Nicolson & Gerd.) Gerd. & Trappe	MN 414D	USA	INVAM
G. gigantea	NC 150	USA	INVAM
G. gigantea	WV 932	USA	INVAM
G. gigantea	NC 199A	USA	INVAM
G. margarita W.N. Becker & I.R. Hall	MAFF 52	Japan	MAFF
G. margarita	MAFF 54	Japan	MAFF
G. margarita	BEG 34	New Zealand	BEG/UNITO
G. margarita	BR 444	Brazil	INVAM
G. rosea T.H. Nicolson & N.C. Schenck	UT 102	USA	INVAM
G. rosea	MAFF 62	Japan	MAFF
G. rosea	BR 151 A	Brazil	INVAM
G. rosea	DAOM 194757	NA	NA
Scutellospora calospora (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders	BEG 32	UK	BEG
S. calospora	HDAM-3	na	na
Scutellospora sp.	W 3485	UK	C. Walker

[a] Species used in the test with species-specific primers (in bold). [b]INVAM: International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi, USA; UFPE: Universidade Federal de Pernambuco, Departamento de Micologia, Brasil; MAFF: Ministry of Agriculture, Forest and Fisheries, Japan; BEG European Bank of Glomales, France; UNITO: Università di Torino, Dipartimento di Biologia Vegetale, Italy; na: information not available.

The 28G1 and 28G2 primers (Silva et al. 2006) were used to amplify the partial LSU rDNA region. NS1, NS2, NS3, NS4, NS5 and NS8 were used to obtain the SSU rDNA amplicons (White et al. 1990).

PCR reactions were carried out in a volume of 50 µl, containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.1 mM $MgCl_2$, 0.01% gelatin, 200 µM each dNTPs, 1 µM of each primer and 2 units of REDTaq™ DNA polymerase (SIGMA) (Sigma-Aldrich, Milan, Italy). Cycling parameters were 45 s at 94°C, 1 min at 55°C, 1 min at 72°C for 40 cycles, with a final elongation of 7 min at 72°C followed the last cycle. The amplicons were visualized on 1.5% agarose gel with ethidium bromide. The amplified products for SSU and LSU rDNA were purified with a QIAquick kit (Qiagen S.p.A., Milan, Italy) following the manufacturer's instruction and sequenced (accession numbers are listed in Table 1). Sequencing was made by GeneLab (Roma, Italy).

Sequence alignment and phylogenetic analysis

Prior to phylogenetic analysis, a BLASTn query of the National Center for Biotechnology information databases demonstrated that sequences obtained from AMF species were affiliated with the genus *Gigaspora*.

Sequences of 15 *Gigaspora* and 11 *Scutellospora* isolates (some obtained from GenBank) were selected to align SSU and LSU rDNA (Table 1) using Clustal X (Larkin et al. 2007) and edited with BioEdit (Hall 1999).

For phylogenetic analyses and tree construction, neighbor joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) analysis with 1,000 bootstrap replications were performed using PAUP4 (Phylogenetic Analysis Using Parsimony vers. 4, Swofford 2003). NJ and ML analyses were performed using parameters obtained from ModelTest 3.7 (Posada & Crandall 1998). *Scutellospora calospora* and *Scutellospora* sp. were used as outgroup.

Results

Morphological observations of isolates FL 927 and BR 444

Isolates FL 927 ('*G. albida*') and BR 444 ('*G. margarita*') had morphological characters similar to other *Gigaspora* species: spore wall thickness [9.6–(12.2)–14.4 µm and 12–(13.9)–16.8 µm, respectively] and spore diameter [230–(282)–360 µm and 250–(320)–384 µm, respectively]. However, the '*G. margarita*' BR 444 spores were hyaline/white to yellow and lacked the characteristic green (*G. albida*) or pink (*G. rosea*) coloration observed in reference isolates *G. albida* BR 601 and *G. rosea* UT 102. All isolates of '*G. margarita*' BR 444 and *G. albida* BR 601 contained endobacteria, which were absent in all isolates of *G. rosea* and '*G. albida*' FL 927.

Testing species-specific primers for taxa of *Gigaspora*

Eleven *Gigaspora* isolates were screened for PCR amplification of a short partial nuclear ITS rDNA fragment (385bp) with species-specific primers (Table 1). The primers GiITS1+GiITS2 amplified only the *G. margarita* species, excluding isolate '*G. margarita*' BR 444, which did not amplify at all (Fig. 1A). The combination of the species-specific primer constructed for *G. rosea* (GiR3) with GiITS1 amplified not only the isolates of *G. rosea*, but also '*G. albida*' FL 927 and showed a slight reaction to the DNA from spores of *G. albida* BR 601 and '*G. margarita*' BR 444 (Fig. 1B), while the GiITS1+ALB1 primer pair amplified only *G. albida* BR 601 and '*G. margarita*' BR 444 (Fig. 1C). These results support a misidentification for '*G. albida*' FL 927 and '*G. margarita*' BR 444.

Phylogenetic analysis of *Gigaspora* from SSU and LSU rDNA

In the SSU rDNA phylogenetic analyses (NJ, MP and ML), the genus *Gigaspora* was supported as a monophyletic group with 100% of the bootstrap value (Fig. 2). Three subclades were observed in the genus, the first grouping '*G. margarita*' BR 444 with all isolates of *G. albida* and *G. rosea*. In the second cluster just *G. gigantea* was present and in the last group all isolates of *G. margarita* (except '*G. margarita*' BR 444) appeared together with *G. decipiens*.

In the tree constructed with the LSU rDNA, *Gigaspora* was monophyletic with high bootstrap support (Fig. 3). All isolates of *G. margarita* (except

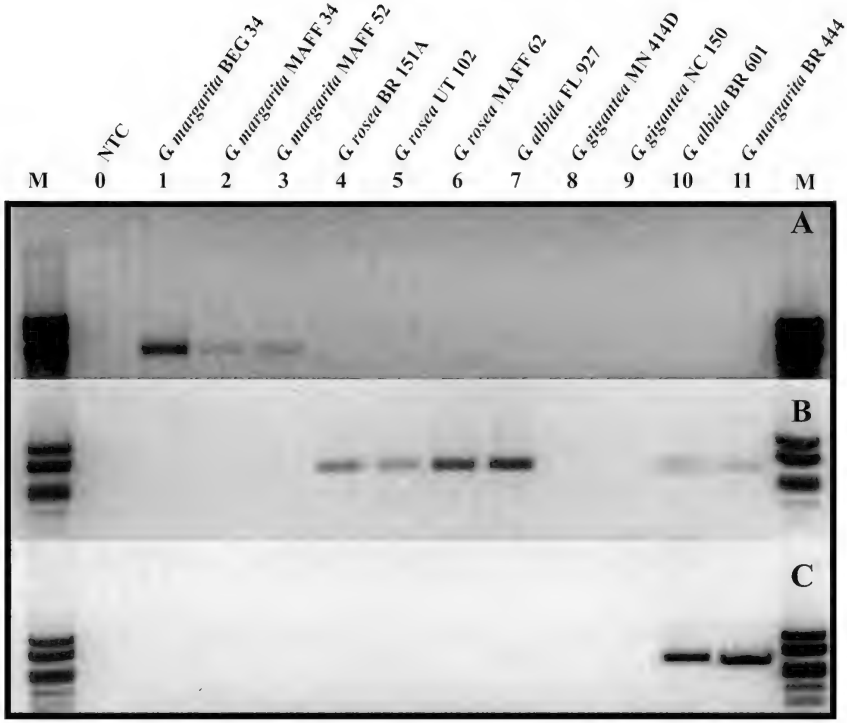

FIG. 1. Agarose gels (1.5%) with PCR products of the *Gigaspora* spp. Different combinations of primers were used: A) GiITS1-GiITS2; B) GiITS1-GiR3; C) GiITS1-ALB1. M = pUC 18 digested with *Hae* III. NTC = no template control.

'*G. margarita*' BR 444) clustered together. Two isolates of *Gigaspora gigantea* grouped in the same clade, *Gigaspora albida* BR 601 and '*Gigaspora margarita*' BR 444 clustered together and *Gigaspora rosea* UT 102 was close to '*Gigaspora albida*' FL 927. Based on SSU and LSU rDNA analyses '*Gigaspora margarita*' BR 444 has been misidentified.

Discussion

Due to the low morphological variation amongst *Gigaspora* species (Bentivenga & Morton 1995), some papers have reported species misidentification (Bago et al. 1998, Souza et al. 2004, Lanfranco et al. 2001). These studies also report the usefulness of molecular approach as an identification tool. Based on sequences of SSU rDNA and isozyme analysis, Bago et al. (1998) divided *Gigaspora* into three subgeneric groups: the first with *G. margarita* and *G. decipiens*, the second with *G. albida* and *G. rosea*, and the third with *G. gigantea*. In this paper these same groups were observed in the phylogeny generated by

FIG. 2. Phylogenetic reconstruction of the *Gigaspora* obtained from SSU rDNA sequences (~1700 bp). The NJ and ML analyses were performed with HKY85 + I substitution model. Bootstrap values (in %) are from neighbor joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) analyses (1000 bootstraps), respectively. Only topologies with bootstrap values of at least 50% are shown. (Consistency Index = 0.88; Retention Index = 0.93).

SSU rDNA, and just '*G. margarita*' BR 444 was out of the correct clade. In the LSU rDNA tree, the isolate BR 444 also grouped together with *G. albida* BR 601, whereas the isolate FL 927 (identified as *G. albida*) was clustered with *G. rosea*. Bago et al. (1998) observed that the isolates BR 444, identified initially as *G. margarita*, and FL 927 (described as *G. albida*), should instead be ascribed to *G. albida* and *G. rosea*, respectively. The PCR amplification tests with species-specific primers in this study also indicated that these two isolates should be reclassified as *G. albida* and *G. rosea*. Nevertheless recently Msiska & Morton

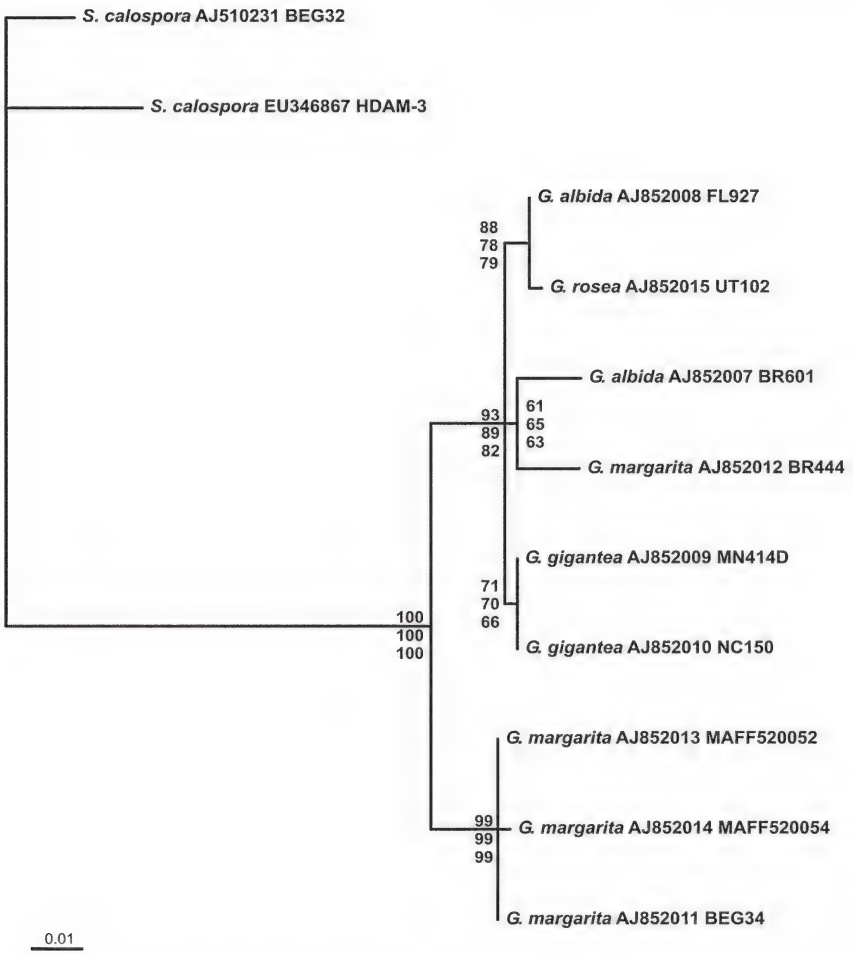

FIG. 3. Phylogenetic reconstruction of the *Gigaspora* obtained from LSU rDNA sequences (~ 450 bp). The NJ and ML analyses were performed with GTR substitution model. Bootstrap values (in %) are from neighbor joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) analyses (1000 bootstraps), respectively. Only topologies with bootstrap values of at least 50% are shown. (Consistency Index = 0.93; Retention Index = 0.93).

(2009), based on β-tubulin gene, reported that '*G. margarita*' BR 444 did not group with *G. albida* or *G. rosea*, but with *G. decipiens*.

Using PCR-DGGE analysis, Souza et al. (2004) already observed, as we confirmed here, that the FL 927 isolate, originally identified as *G. albida,* should be ascribed to *G. rosea*, indicating identification problems. Endobacteria are usually observed in glomerospores of *Gigaspora* species, with the exception of

G. rosea isolates. Bacteria were not found in '*Gigaspora albida*' FL 927, further substantiating the need for reclassification.

Van Tuinen et al. (1998) were the first to design specific primers for *Gigaspora* species (*G. rosea*) that amplify a region of the LSU rDNA. Because these authors tested cross-amplification only with species of other AMF genera, it is not possible to establish whether the primers amplify other *Gigaspora* species. Currently there are primers specific for two *Gigaspora* species (*G. margarita* and *G. rosea*); nevertheless, we report that the reverse primer GiR3 (Lanfranco et al. 2001) also amplifies *G. albida* isolates. As Lanfranco et al. (2001) did not use *G. albida* and *G. decipiens* in their analyses with species-specific primers, it was not possible to establish how specific the primers are to *G. rosea* and *G. margarita*. Of the five known *Gigaspora* species (Bentivenga & Morton 1995), only *G. decipiens* was not used in this work.

In summary, our results show that different specific PCR primer pairs and phylogenetic studies can help discriminate among *Gigaspora* species with similar spore morphologies and support the utility of these primers and phylogenetic analysis for solving problems in identifying groups of species in *Gigasporaceae* with a low morphological variation.

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Contribution to the taxonomy of *Bovista* in Mexico

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Abstract — Specimens of the genus *Bovista* from eight Mexican herbaria (CESUES, ENCB, FCME, HEMIM, IBUG, IZTA, MEXU, XAL) were examined and studied, and fifteen taxa were identified: *Bovista aestivalis, B. brunnea, B. dermoxantha, B. dominicensis, B. fusca, B. grandipora, B. graveolens, B. heterocapilla, B. leucoderma, B. oblongispora* var. *longispora*, *B. oblongispora* var. *oblongispora, B. plumbea, B. pusilla, B. sempervirentium,* and *B. tomentosa*. The new information obtained indicates a wider distribution for *B. aestivalis, B. dermoxantha, B. fusca, B. graveolens, B. leucoderma, B. oblongispora* var. *longispora, B. pusilla,* and *B. tomentosa*. New records for the Mexican mycobiota are: *B. dominicensis, B. grandipora, B. heterocapilla, B. oblongispora* var. *oblongispora,* and *B. sempervirentium.*

Key words — *Basidiomycota,* taxonomy, gasteroid fungi

Introduction

The genus *Bovista* Pers. is characterized by globose to pyriform basidiomes with/without a short pseudostipe and with/without mycelial cords (Pegler et al. 1995) and a thin, fragile, scaly exoperidium. On maturing the basidiome may form plates, areolae, verrucae, or scales microscopically composed of hyphal elements, sphaerocysts, claviform cells (Kreisel 1967), or mycosclereids (Calonge et al. 2005). The endoperidium is persistent, in consistency papyraceous, pseudopapyraceous, or rigid, smooth and generally shiny or with a metallic luster (Coker & Couch 1928), although this does not distinguish *Bovista* from other related genera. According to Smith (1951), spores are

smooth or ornamented. Shape varies, from globose, subglobose to oblong, with a pedicel, which may be short or long, straight or curved, corresponding to the sterigma of the basidium. The subgleba, present only in some species, has a filamentous, compact appearance and is generally reduced in size and not well developed. There are three types of capillitium: *Lycoperdon*, *Bovista,* and intermediate (Calonge 1998; Krüger et al. 2001).

Bovista, traditionally placed in *Lycoperdaceae* but currently classified in *Agaricaceae* (Kirk et al. 2008), is divided into two subgenera based on capillitial type. Taxa characterized by a *Lycoperdon* or intermediate capillitium are placed in subg. *Globaria*, while those with either a *Bovista* or heteromorphic (*bovista-* + intermediate) capillitium belong in subg. *Bovista*. These subgenera are further divided into sections and series based on capillitium type, absence or presence of capillitial pores, and presence or absence of a subgleba (Kreisel 1967).

In Mexico few researchers have addressed the taxonomy of *Bovista* (Herrera 1959); the genus is generally included in general gasteromycete treatments (Salcedo & Herrera 1966, Guzmán & Herrera 1969, 1973, Rodríguez & Herrera 1970, Urista et al. 1985, Esqueda et al. 1990, 1996, 1998, Pardavé 1991, Pérez-Silva et al. 1994, Calonge et al. 2004a,b, Ochoa & Moreno 1996, 2006). *Bovista* has been confused with *Lycoperdon* Pers., *Disciseda* Czern., *Calvatia* Fr., *Vascellum* F. Šmarda, *Bovistella* Morgan, and *Calbovista* Morse ex M.T. Seidl, owing to shared macro- and microscopic characteristics. There are, however, differences that allow them to be quite easily separated. Confusion surrounding the correct identification of species has prompted this revision of *Bovista* with the aim of contributing to the knowledge of the genus in Mexico.

Materials & methods

Herbarium material was examined from CESUES (Centro de Estudios Superiores del Estado de Sonora, Unidad Hermosillo), ENCB (Escuela Nacional de Ciencias Biológicas, IPN), FCME (Facultad de Ciencias, UNAM), HEMIM (Herbario Micológico de Morelos, UAEM), IBUG (Instituto de Botánica, Universidad de Guadalajara), IZTA (Facultad de Estudios Superiores campus Iztacala, UNAM), MEXU (Instituto de Biología, UNAM), and XAL (Instituto de Ecología, A.C., Unidad Xalapa).

Macroscopic characters were transcribed from specimen labels or recorded directly from the basidiomes. For microscopic examination, temporary preparations were made using 5% potassium hydroxide (KOH) for observing and measuring basidiospores and capillitial elements with an optical microscope (OM). Lactophenol cotton blue was used for observing the paracapillitium. Material was also prepared for observation using a scanning electron microscope (SEM) in order to properly describe spore ornamentation and perforations in the hyphal walls of the capillitium.

The keys of Kreisel (1967), Demoulin & Marriot (1981), Calonge (1992, 1998) and Pegler et al. (1995) were mainly used for species identification.

Results

Of the 15 *Bovista* taxa identified and included in the taxonomic key, 9 represented subgenus *Globaria* (*B. aestivalis*, *B. dermoxantha*, *B. dominicensis*, *B. grandipora*, *B. heterocapilla*, *B. oblongispora* var. *longispora*, *B. oblongispora* var. *oblongispora*, *B. pusilla*, *B. sempervirentium*) and 6 subgenus *Bovista* (*B. brunnea*, *B. fusca*, *B. graveolens*, *B. leucoderma*, *B. plumbea*, *B. tomentosa*). Five taxa are new records for the Mexican mycobiota and are described below: *B. dominicensis*, *B. grandipora*, *B. heterocapilla*, *B. oblongispora* var. *oblongispora*, and *B. sempervirentium*.

Key to the taxa of Bovista recorded from Mexico

1. Basidiomes with intermediate or *Lycoperdon* capillitium 2
1'. Basidiomes with *Bovista* type capillitium 11

2. Intermediate type capillitium 3
2'. *Lycoperdon* type capillitium 7

3. Basidiomes with ellipsoid spores, short pedicels 4
3'. Basidiomes with globose spores 5

4. Capillitium with pores ..... *B. oblongispora* var. *longispora*
4'. Capillitium pores absent ..... *B. oblongispora* var. *oblongispora*

5. Heteromorphic capillitium 6
5' Intermediate capillitium 7

6. Spores with pedicels ≤ 11 μm ..... *B. pusilla*
6'. Spores with pedicels ≤ 17 μm ..... *B. heterocapilla*

7. Echinulate spores with pedicels ≤ 34 μm ..... *B. dominicensis*
7'. Spores with small verrucae 8

8. Capillitium with branches 9
8'. Capillitium without branches 10

9. Capillitium with amphiseptal branches, numerous pores, giving a vacuolate appearance ..... *B. grandipora*
9'. Capillitium with subseptal branches, with pores ..... *B. dermoxantha*

10. Exoperidium verrucae made of sphaerocysts ..... *B. aestivalis*
10' Exoperidium verrucae made of hyphal elements ..... *B. sempervirentium*

11. Basidiomes medium to large, > 55 mm 12
11' Basidiomes small to medium, ≤ 55 mm 13

12. Spores subglobose to ovoid, pedicels straight ..... *B. fusca*
12'. Spores globose to subglobose, pedicels curved ..... *B. graveolens*

13. Capillitium with pores 14
13'. Capillitium pores absent, endoperidium lead colored ..... *B. plumbea*

14. Spores ovoid ..... *B. tomentosa*
14'. Spores globose to subglobose 15
15. Capillitium with thick walls, LH = 0.2–0.5 ..... *B. brunnea*
15'. Capillitium with moderately thin walls, LH = 0.5–0.7 ..... *B. leucoderma*

Taxa studied

Subgenus *Globaria*

Bovista aestivalis (Bonord.) Demoulin, Beih. Sydowia 8: 143, 1979. FIGS 1–2

SPECIMENS EXAMINED: **MÉXICO. HIDALGO:** MINERAL DEL CHICO MUNICIPALITY, near Las Ventanas, El Chico National Park, October 5, 1980 *G. Guzmán 19099* (ENCB). **JALISCO:** SAN DIEGO DE ALEJANDRÍA MUNICIPALITY, San Diego de Alejandría-San Julián Highway, 7.5 km from the town of San Diego de Alejandría, August 30, 2000 *O. Rodríguez 2332* (IBUG). **ESTADO DE MÉXICO:** Near Amecameca, August 31, 1969 *I. Uribe 11* (ENCB); Near La Marquesa, Miguel Hidalgo National Park, July 5, 1963 *E. González 12* (ENCB); ATIZAPAN DE ZARAGOZA MUNICIPALITY, Sayavedra Farm subdivision, June 18, 2000 *A. López-Villalobos 24* (XAL). **OAXACA:** San Pedro Macuiltianguis, June 15, 1984 *A. González* (XAL).

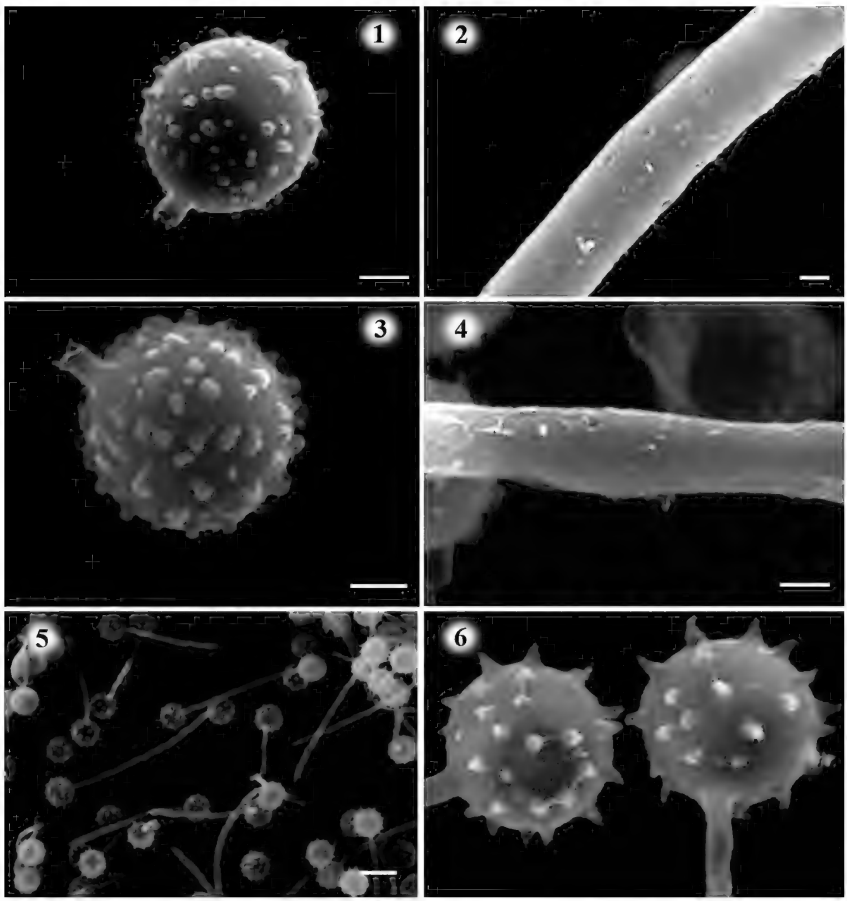
COMMENTS — This species is characterized by a compact subgleba, evident or barely developed. Unlike *Bovista dermoxantha, B. aestivalis* has an intermediate type of capillitium and under the OM the spores show an ornamentation that is not very evident. Pegler et al. (1995) mention that it is a very polymorphic species and thus taxonomically difficult to identify.

Bovista aestivalis has been cited for Baja California (Ochoa & Moreno 2006), Chihuahua (Moreno et al. 2010), Estado de México, Oaxaca and Veracruz (Calonge et al. 2004b). This study extends its distribution range to the states of Hidalgo and Jalisco.

Bovista dermoxantha (Vittad.) De Toni, Syll. Fung. 7: 100, 1888. FIGS 3–4

SPECIMENS EXAMINED: **MÉXICO. BAJA CALIFORNIA:** km 2, close to the turnoff from Ensenada to the San Felipe highway, on the way to Hanson Lagoon, in the foothills of the Juárez range, March 02, 1984 *G. Guzmán 24326-A* (XAL). **CHIHUAHUA:** TEMÓSACHI MUNICIPALITY, Nabogame, July 31, 1987 *J. E. Laferrière 624* (XAL); 65 km NW of Janus, September 20, 1972 *P. Huerta* (ENCB). **HIDALGO:** San Gregorio, north side of Xihuingo Hill, July 26, 1963 *G. Guzmán 3865* (ENCB). **ESTADO DE MÉXICO:** Close to the Guadalupe Dam, July 02, 1967 *G. Guzmán 5862* (ENCB). **SONORA:** km 12 on the Santa Rosa-Yécora highway, August 6, 1989 *A. Aparicio 4* (MEXU 22589). **TLAXCALA:** HUAMANTLA MUNICIPALITY, 2 km south of La Malinche mountain, July 19, 1987 *Mata 217* (XAL). **VERACRUZ:** Montepío, June 19, 1969 *R. Singer & T. Herrera* (MEXU 6897); IZTACZOQUITLÁN MUNICIPALITY, in the region of Metlac Gorge, August 09, 1995 *P. Navarro 1042*; September 13, 1996 *P. Navarro 1095* (XAL).

COMMENTS — Pegler et al. (1995) mention that this species is close to *B. aestivalis* but differs by its conspicuously ornamented spores and the *Lycoperdon* type capillitium.



Figs. 1–6. *Bovista aestivalis*: 1. Spores. 2. Pitted capillitium. *Bovista dermoxantha*: 3. Spores. 4. Pitted capillitium. *Bovista dominicensis*: 5. Spores. 6. Spores ornamentation detail. Scale bars: 1–4, 6 = 1 µm; 5 = 5 µm.

Bovista dermoxantha has been reported for the states of Baja California (Calonge et al. 2004b; Ochoa & Moreno 2006), Chihuahua, Tlaxcala and Veracruz (Calonge et al. 2004b). This study extends its distribution range to Hidalgo, Estado de México and Sonora.

Bovista dominicensis (Massee) Kreisel, Feddes Repert. 69: 202, 1964. Figs 5–6

Basidiome 12–37 mm, globose to flattened-globose, exoperidium forms small pyramidal scales like spines, blackish-brown to greyish in color, formed by sphaerocysts that are reddish-brown in KOH; endoperidium whitish, of intertwined hyaline hyphae. Gleba white to olive green. Subgleba compact.

Lycoperdon type capillitium, hyphae thin walled, 4–5 µm, light brown in color, pores absent, barely branched, septa scarce. Paracapillitium absent. Spores globose, 4–5 µm, equinulate, hyaline. Pedicel 13–30(–34) × 1 µm, straight, hyaline.

Ecology & distribution — Gregarious, humicolous in cloud forests, *Pinus–Quercus* in transition with cloud forest and tropical vegetation with columnar cacti, at an altitude of 1270–1550 m.

Specimens examined: **México. Puebla:** La Magdalena Farm, S of Teziutlán, October 14, 2004 *E. Gándara 1161*. **Sinaloa:** El Fuerte road to stones with hieroglyphics, after the El Fuerte River, August 24, 2004 *G. Guzmán 36091*. **Veracruz:** Surroundings of the CONECALLI-DIF social services building, km 2.5 Xalapa-Coatepec Old Highway, September 7, 1993 *D. Fernández 38*; Xico Municipality, road from Xico to Matlalapa, before arriving at Xico Viejo, September 3, 2000 *D. Jarvio 677*; Banderilla Municipality, La Martinica Hill, July 01, 2004 *V. Ramírez-Cruz 94*; La Martinica Hill, August 21, 2006 *G. Guzmán 36569*; Coatepec Municipality, Zoncuantla, royal road from Xalapa to Coatepec near km 6 of the Xalapa-Coatepec Old Highway (residence), September 18, 1998 *G. Guzmán 32468*; September 20, 1998 *G. Guzmán 32482*; September 29, 1998 *G. Guzmán 32666*; December 30, 1998 *G. Guzmán 32771*; June 24, 1999 *G. Guzmán 32995*; June 27, 1999 *G. Guzmán 33022*; June 01, 2000 *G. Guzmán 33495*; July 13, 2003 *G. Guzmán 35488*; October 03, 2003 *G. Guzmán 35568*; November 02, 2003 *G. Guzmán 35704*; October 02, 2004 *G. Guzmán 36119*; *G. Guzmán 36122*; October 05, 2004 *G. Guzmán 36125*; *G. Guzmán 36127*; October 07, 2004 *G. Guzmán 36133*; *G. Guzmán 36277*; July 10, 2005 *G. Guzmán 36228*; July 26, 2005 *G. Guzmán 36311*; *G. Guzmán 36328*; October 23, 2005 *G. Guzmán 36367*; July 02, 2006 *G. Guzmán 36440*; September 15, 2006 *G. Guzmán 36678*; Xalapa Municipality, F.J. Clavijero Botanical Garden, km 2.5 on the Old Highway to Coatepec, June 03, 1995 *García-Velázquez 779*; July 03, 1995 *García-Velázquez 780*; July 10, 2003 *F. Escalona 196*; September 30, 2004 *E. Gándara 892*; October 07, 2004 *E. Gándara 932*; *V. Ramírez-Cruz 189* (XAL).

Comments — *Bovista dominicensis*, is characterized microscopically by globose and echinulate spores with long pedicels from 13 up to 34 µm. Macroscopically it can be confused with *Lycoperdon* because the basidiome is pyriform owing to the presence of a pseudostipe.

Part of the material examined had been identified as *B. longissima* Kreisel and thus reported as a new record for the Americas (Calonge et al. 2004b). However, *B. longissima* has verrucose spores and pedicels ≤ 38 µm long and to date it has been recorded only from Japan (Kreisel 1967). Spore ornamentation places it in *B. dominicensis*, as it is the same as that in the SEM images shown in the study by Calonge et al. (2005). Although those authors describe the spores as verrucose, Kreisel (1967) described them with fine verrucae to delicate spines. The pedicels of the examined specimens were longer (≤ 34 µm) than those reported by Kreisel (10–21 µm) and Calonge et al. (20 µm).

Worldwide, *B. dominicensis* has been reported for Brazil (Kreisel 1967, Homrich 1969 [as *Lycoperdon dominicensis*], Trierveiler et al. 2010), the United States of America, the Dominican Republic (Kreisel 1967), Colombia (Guzmán

et al. 2004), and Costa Rica (Calonge et al. 2005). This is the first report of *B. dominicensis* for Mexico, where it has been recorded from the states of Puebla, Sinaloa and Veracruz.

Bovista grandipora Trierv.-Per., Kreisel & Baseia, Mycotaxon 111: 415, 2010. FIGS 7–8

Basidiome 10–12 mm in diameter, globose. Exoperidium white, breaking into small scales, furfuraceous in appearance, made up of short fragments of hyphae, orange-red color in KOH. Endoperidium greyish brown to dark brown. Gleba white to mustard yellow. Subgleba absent. Mycelial cord radicant.

Lycoperdon type capillitium, with amphiseptal septa, pores abundant, giving a vacuolate appearance. Globose spores, 4–5 µm, verruculose, greenish-yellow. Short pedicel, 0.5–1 µm, hyaline.

ECOLOGY & DISTRIBUTION —Terricolous, gregarious in the grass. Found in open *Juniperus deppeana* forest and in tropical vegetation at altitudes of 2500 and 1500 m, respectively.

SPECIMENS EXAMINED: **MÉXICO. JALISCO:** SAN PEDRO TESISTÁN MUNICIPALITY, highway to Azuayo, W side of the Chapala Lagoon, July 07, 1974 *G. Guzmán 11582-B* (ENCB); ZAPOPAN MUNICIPALITY, 2 km NW of La Venta, September 27, 1970 *D. García 452* (IBUG). **PUEBLA:** Santa Cruz, between San Martín Texmelucan and La Blanca, August 25, 1956 *T. Herrera* (MEXU 901).

COMMENTS — The specimens examined coincide with the description of *Bovista grandipora* given by Trierveiler-Pereira et al. (2010), Microscopically, *B. grandipora* is characterized by a capillitium of the *Lycoperdon*-type with numerous pores, 0.5–2.4 µm, round to ellipsoid or irregularly shaped, and globose, verruculose with short pedicel (0.5–1 µm) basidiospores.

Worldwide, this species has been recorded for Brazil, Cuba, Dominican Republic, India, Japan, Nepal, Puerto Rico and Spain (Trierveiler-Pereira et al. 2010). This is its first record for Mexico, specifically for the states of Jalisco and Puebla.

Bovista heterocapilla Kreisel, Beih. Nova Hedwigia 25: 224, 1967. FIGS 9–11

Basidiome globose, subglobose to turbinate. Endoperidium papyraceous, smooth, brown in color, made of intertwined hyphae. Exoperidium is white, ephemeral, persistent small spines (spinulose), blackish-brown, made up of sphaerocysts. Subgleba absent or if present, compact, olive brown to dark brown.

Bovista type or heteromorphic capillitium in the center of the gleba, hyphae with pseudosepta frequent, true septa and pores absent. Spores 5–6 µm, verrucose, pedicellate. Pedicel 8–17 µm, straight, hyaline.

ECOLOGY & DISTRIBUTION — Gregarious, terricolous in *Abies, Abies-Quercus* and cloud forest at an altitude of 2330 to 2800 m.

Specimens examined: **MÉXICO. Distrito Federal:** Contreras, Cerro Tarumba, October 21, 1956 *T. Herrera* (MEXU 1426). **Hidalgo:** El Chico National Park, Las Ventanas, September, 1970 *R. Ramos 105* (ENCB); El Chico National Park, October 25, 1975 *R. Hernández* (MEXU 12871); Piedras Voladas, Real del Monte, June 13, 1982 *E. Pérez*-Silva & *R. Hernández* (MEXU 17283); Pueblo Nuevo, October 08, 1975 *E. Pérez-Silva* (MEXU 11312). **Estado de México:** Amecameca, September 18, 1963 *A. Nájera* (MEXU 2761); La Marquesa-Tenango Highway km 6, October 05, 1975 *R. Lamothe* & *E. Pérez-Silva* (MEXU 10163); La Marquesa, October 21, 1973 *R. Lamothe* & *E. Pérez-Silva* (MEXU 8442); Purificación NE of Texcoco, September 05, 1976 *G. Guzmán 16477* (ENCB).

Comments — This species is characterized by a *Bovista* type capillitium; heteromorphic in some specimens, i.e., of the intermediate+*Bovista* type. Although Kreisel (1967) mentions that it has the *Lycoperdon* type of capillitium, the characteristics of the hyphae and the spores match those cited for *B. heterocapilla*.

One species close to *B. heterocapilla* is *B. albosquamosa* Kreisel. These are distinguished by the characteristics of the exoperidium and the spore ornamentation. In *B. heterocapilla* the exoperidium is uniformly granular-verrucose and the spores are verrucose, while in *B. albosquamosa* the exoperidium is furfuraceous areolate and the spores are dotted or verruculose (Kreisel 1967).

Bovista heterocapilla has been reported only for Jamaica (Kreisel, 1967). This is the first record for Mexico.

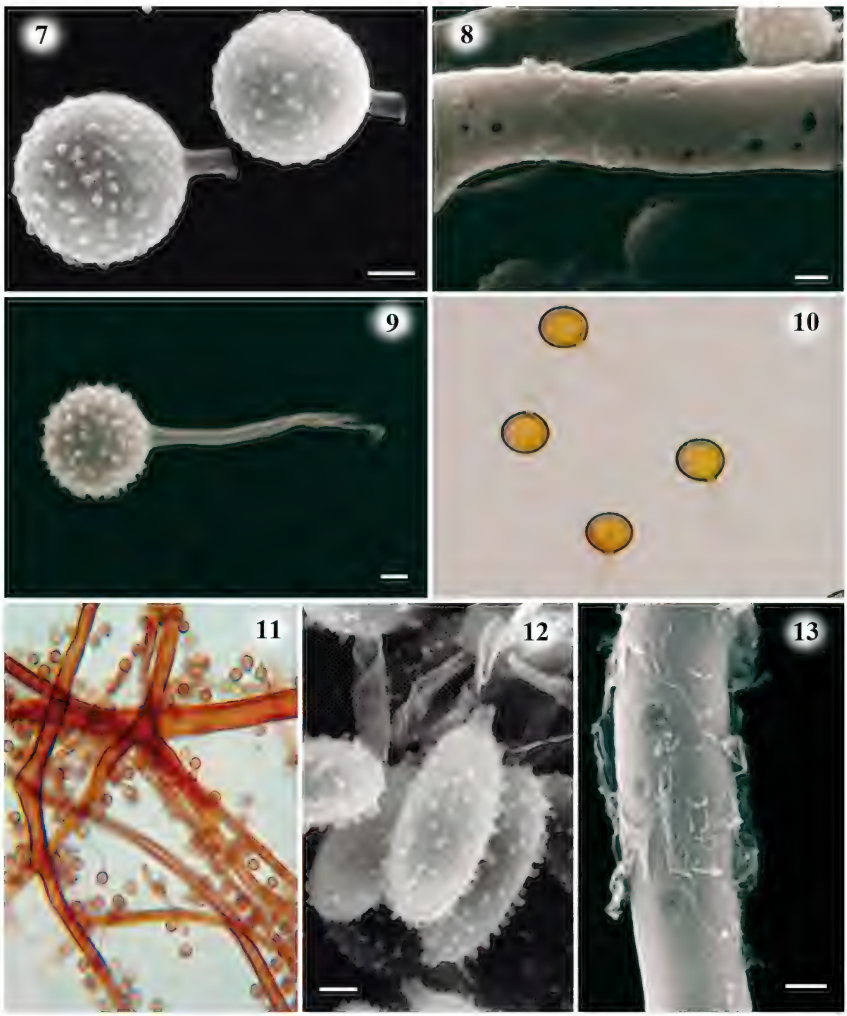
Bovista oblongispora var. ***longispora*** (Kreisel) A. Ortega & Buendía, Cryptogamie, Mycol. 6: 285, 1985. Figs 12–13

Basidiome 13–20 × 10–15 µm, subglobose to slightly pyriform; when young, the exoperidium is reddish in color, later it disintegrates, leaving reddish-brown scaly remains, exposing an ochraceous endoperidium, shiny and papyraceous in consistency. Gleba whitish, yellowish to dark chocolate brown, fleshy-alveolate to powdery in consistency. Subgleba present.

Intermediate type capillitium, hyphae 4–5(–6) µm, pores moderately frequent, wall thin, septate, with dichotomous branching. Paracapillitium absent. Spores 5–7 × 3–4 µm, ellipsoid to cylindrical, verrucose; verrucae irregularly distributed. Pedicel 1–2.5 µm, occasionally up to 8 µm.

Ecology & distribution — Gregarious, terricolous in *Quercus–Pinus* forest, cloud forest, pastures and coffee plantations at altitudes of 1250–2300 m.

Specimens examined: **MÉXICO. Puebla:** Close to the Ocochiguaya Gorge, E side of Chignahuapan, July 12, 1975 *Herrera, Hernández, Cruz* & *Guzmán* (MEXU 25336). **Sonora:** Yécora Municipality, km 3.4 along the Yécora- Las Cabañas road, September 12, 1996 *M. Esqueda, A. Armenta, A. Núñez* & *R. Santos* (CESUES 3181); September 13, 1996 (CESUES 3013); Ónavas Municipality, km 204.5 on the Hermosillo-Yécora road, October 06, 1995 *E. Pérez, T. Herrera, M. Esqueda, A. Armenta, A. Núñez, R. Rodríguez* & *R. Santos* (CESUES 2107). **Veracruz:** Highway to Fortín, 10 km from



Figs. 7–13. *Bovista grandipora*: 7. Spores. 8. Pitted capillitium. *Bovista heterocapilla*: 9. Spore under SEM. 10. Spores under OM ×1600. 11. Capillitium and spores under OM ×400. *Bovista oblongispora* var. *longispora*: 12. Spores. 13. Pitted capillitium. Scale bars = 1 μm.

Huatusco, July 20, 1983 *S. Chacón 1189* (XAL); highway to Fortín, Tepampa turnoff, 10 km from Huatusco, July 27, 1983 *G. Guzmán 23527* (XAL); Coatepec Municipality, Zoncuantla, close to the road to Coatepec, August 15, 1994 *G. Guzmán 30930* (XAL).

Comments — For the identification of this species the criteria of Ortega & Buendía (1985) were applied. They propose that *B. longispora* and *B. oblongispora*

are varieties of a single species. They argue that the difference between the two lies in the respective presence or absence of pores in the capillitium.

In his monograph, Kreisel (1967) treats the two taxa as separate species, stating that *B. longispora* has a *Lycoperdon* type capillitium with pores and compact subgleba, while *B. oblongispora* has an intermediate type capillitium, no pores, and no subgleba.

Bovista oblongispora var. *longispora* has previously been reported from Mexico as *B. longispora* (Calonge et al. 2004b; Esqueda et al. 1996; 1998 and Pérez-Silva et al. 1994).

Bovista oblongispora (Lloyd) Bottomley, Bothalia 4(3): 580, 1948
var. ***oblongispora*** FIGS 14–15

Basidiome globose to subglobose 17–18 mm in diameter. Exoperidium formed by small needles separated by approximately 0.5 mm, comprised of hyphae and sphaerocysts; endoperidium brown in color to dark chocolate brown, membranous. Gleba powdery, yellowish-brown to dark brown in color. Subgleba yellowish-brown, compact.

Intermediate type capillitium, hyphae 6–7(–8) µm, pores absent, wall thin, dichotomous branching. Paracapillitium absent. Basidiospores 5–7 × 2.5–3 µm, oblong to ellipsoid, verrucose, with a regular longitudinal arrangement. Pedicel 0.5 µm, hyaline.

ECOLOGY & DISTRIBUTION — Solitary, terricolous in cloud forest at an altitude of 1300 m.

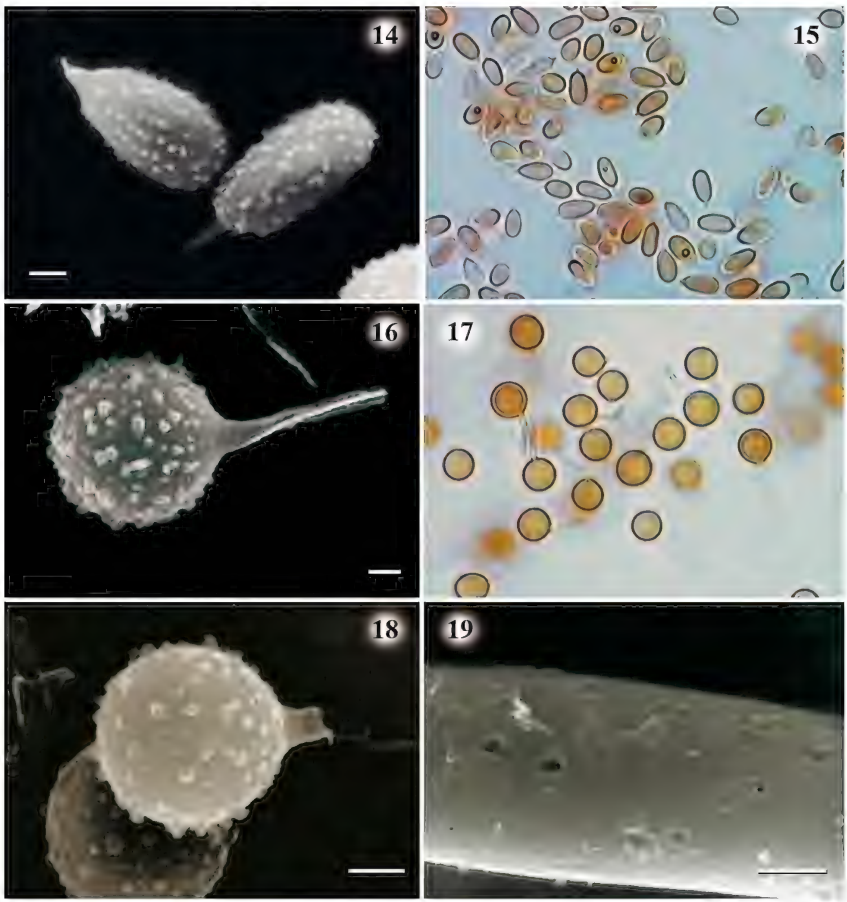
SPECIMEN EXAMINED: **MÉXICO. VERACRUZ:** XALAPA MUNICIPALITY, km 2.5 on the Xalapa-Coatepec Old Highway, Francisco Javier Clavijero Botanical Garden, May 14, 2005 *Gándara 1268* (XAL).

COMMENTS — This variety can be distinguished from *B. oblongispora* var. *longispora* by the absence of pores and the generally predominant ellipsoid shape of the spores. This is the first record for Mexico.

Bovista pusilla (Batsch) Pers., Syn. Meth. Fung. 1: 138, 1801. FIGS 16–17

SPECIMENS EXAMINED: **MÉXICO. JALISCO:** ZAPOTLÁN EL GRANDE MUNICIPALITY, the foothills of Nevado de Colima, Las Víboras track, Floripondio, June 27, 1999 *Montoya-Fuentes 14* (IBUG). **ESTADO DE MÉXICO:** La Marquesa, October 18, 1976 *A. González* (FCME 496); NAUCALPAN MUNICIPALITY, Los Remedios, October 7, 1956 *T. Herrera & O. Sánchez* (MEXU 1416). **SONORA:** YÉCORA MUNICIPALITY, km 258 on the highway from Hermosillo to Yécora, October 5, 1995 *E. Pérez-Silva, T. Herrera, M. Esqueda, A. Armenta, A. Núñez, R. Rodríguez & R. Santos* (CESUES 2073).

COMMENTS — For *B. pusilla,* we do not follow the species concept of Kreisel (1967), who characterizes this species by small basidiomes with a *Lycoperdon*-type capillitium and non-pedicellate spores, characters not seen in the recently examined material. Larsson et al. (2009) amply discuss the taxonomic,



Figs. 14–19. *Bovista oblongispora* var. *oblongispora*: 14. Spores under SEM. 15. Spores under OM ×1000. *Bovista pusilla*: 16. Spores under SEM. 17. Spores under OM ×1000. *Bovista sempervirentium*: 18. Spores. 19. Pitted capillitium. Scale bars = 1 μm.

ecological, and phylogenetic aspects of *B. pusilla* that were considered in the determination of the studied materials: finely warted spores of 4.5–5.5 μm, pedicels of 3–9 μm, heteromorphic capillitium, a temperate distribution, and an association with mosses. These characteristics totally match the studied material.

Following Kreisel's (1967) concept, *B. pusilla* has been cited from seven Mexican federative entities (Guzmán & Herrera 1969; 1973; Rodríguez & Herrera 1970; Urista et al. 1985; Esqueda et al. 1990; 1996; Pérez-Silva et al. 1994); but following the criteria of Larsson et al. (2009), this species is known only from the states of Mexico, Jalisco, and Sonora.

Worldwide, *B. pusilla* sensu Kreisel (1967) is widely distributed in Europe and America and sensu Larsson et al. (2009) is registered from the northern Europe region.

Bovista sempervirentium Kreisel, Beih. Nova Hedwigia 25: 107, 1967. Figs 18–19

Basidiome 10–20 mm in diameter, globose. Exoperidium formed by small furfuraceous verrucae, comprised of hyphal elements. Endoperidium pseudopapyraceous, ochre-brown in color. Gleba is olive brown. Subgleba compact.

Intermediate type capillitium, hyphae 4–5 µm, with small pores, without septa or pseudosepta. Paracapillitium absent. Spores 3.5–5 µm, globose, verruculose, with a short pedicel.

Ecology & distribution — Solitary, terricolous in *Abies* and *Pinus* forest, although Kreisel (1967) reported it for a *Quercus virginiana* forest.

Specimens examined: **MÉXICO. Distrito Federal:** Los Leones Desert, July 02, 1950 *T. Herrera* (MEXU 1438). **Estado de México:** El Carmen Hill, Chimalpa, October 28, 1956 *T. Herrera* & *O. Sánchez* (MEXU 1430).

Comments — The examined specimens match *B. sempervirentium* as described by Kreisel (1967): they have an intermediate type capillitium with small pores but without septa or pseudosepta, verruculose spores with a short pedicel, an exoperidium composed of hyphal elements, and a subgleba.

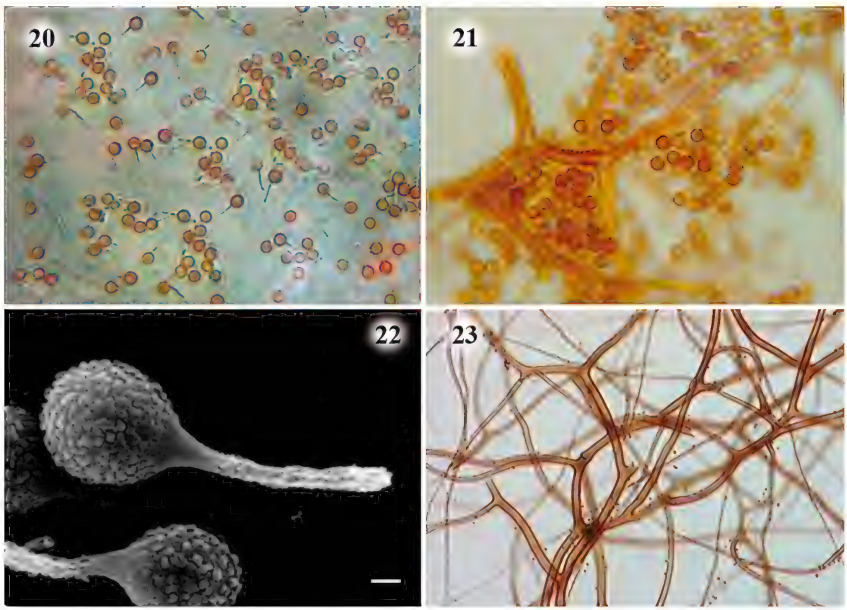
The species has been reported only for the United States of America (Kreisel 1967), thus this is the first record for Mexico, specifically for the Distrito Federal and the Estado de México.

Subgenus *Bovista*

Bovista brunnea Berk., Fl. Nov.-Zel. 2: 189, 1855. Figs 20–21

Specimens examined: **MÉXICO. Distrito Federal:** Los Leones Desert, July 10, 1949 *M. Ruiz-Oronoz & T. Herrera* (MEXU 1418). **Veracruz:** Las Vigas Municipality, Surroundings of El Volcancillo, SW of Encino Gacho, Xalapa-Perote road, November 30, 1991 *F. Tapia 922* (XAL).

Comments — This species is characterized by *Bovista* type capillitium with pores and no septa and a hyphal-vesiculate exostratum. An exoperidium was not observed in the examined material; only some amorphous structures could be seen, possibly owing to the weathering of the specimen. *Bovista brunnea* is placed in the *Globisporae* series, which also includes *B. echinella* Pat., *B. verrucosa* (G.H. Cunn.) G.H. Cunn., and *B. leucoderma*. *Bovista echinella* is distinguished by a hyphal exostratum and a thin papyraceous endoperidium, *B. verrucosa* has a distinctive mycelial cord, and *B. leucoderma* has a white exoperidium that forms small plates on the endoperidium and a capillitium with infrequent septa.



Figs S. 20–23. *Bovista brunnea*: 20. Spores under OM ×500. 21. Capillitium and spores ×600. *Bovista fusca*: 22. Spores. 23. Capillitium under OM ×100. Scale bars = 1 µm.

Herrera (1959) mistakenly cited *B. brunnea* for the Estado de México but later redetermined the collection as *B. dealbata* [≡ *B. leucoderma*] (Herrera 1964, Guzmán & Herrera 1969), and thus the *B. brunnea* record for the state must be discarded. The only authentic previous record from Mexico is from Veracruz (Calonge et al. 2004b).

Bovista fusca Lév., Ann. Sci. Nat., Bot., sér. 3, 5: 303, 1846. Figs 22–23

Representative specimens examined (total collections = 103): **México. Distrito Federal:** Monte Alegre, Ajusco Mountains, September 20, 1980 *E. Aguirre* (MEXU 17044). **Durango:** Pueblo Nuevo Municipality, Las Bayas Property, Los Otates stream, July 16, 2009 *S. Bautista* & *E. Aguirre* (MEXU 26301). **Guerrero:** Chichihualco Municipality, km 8.5 between El Carrizal and Atoyac, *M. Robledo 61* July 12, 1980, (FCME 986). **Hidalgo:** Piedras Voladas, Real del Monte, June 13, 1982 *E. Pérez-Silva* & *R. Hernández* (MEXU 17282). **Jalisco:** Ciudad Guzmán Municipality, Nevado de Colima Mountain foothills, El Floripondio, September 25, 1992 *V. Chaparro 3* (IBUG, MEXU 25378). **Estado de México:** Jalatlaco Municipality, km 13.5 on the Jalatlaco-El Ajusco Highway, August 4, 1986 *A. Calderón* & *E. Aguirre* (MEXU 21594). **Michoacán:** Mariposa Monarca Sanctuary, near Angangueo, February 5, 1981 *S. Acosta 584* (ENCB). **Morelos**: Zempoala Lagoons, July 27, 1985 *A. Calderón* (MEXU 19857). **Oaxaca:** 3 km SE of Plan de Guadalupe, La Cañada, June 13, 1991 *Pérez-Silva* et

al. (MEXU 22788). **Puebla:** Chignahuapan Municipality, Apizaco-Chignahuapan Highway, Cieneguilla Larga, July 13, 2000, *F. Tapia 2050* (XAL). **Tlaxcala:** San Salvador, November 13, 1983 *C. Martínez* (IZTA 2540). **Veracruz:** Rafael Lucio Municipality, Santa Bárbara Farm, 10 km NE of the Xalapa-La Joya Highway, July 8, 1998 *F. Ramírez-Guillén 100* (XAL).

Comments — This species is characterized macroscopically by a papyraceous shiny peridium, especially in mature specimens; on maturing the basidiomes separate easily from the substrate. Macroscopically it can be confused with *B. graveolens*, however the difference lies mainly in the shape of the pedicels. Some authors have confused *B. fusca* with *B. nigrescens* Pers. (Calonge et al. 2004b), although that species occurs only in Europe; Calonge et al. (2005) verified that the XAL specimens earlier identified as *B. nigrescens* represent instead *B. fusca.*

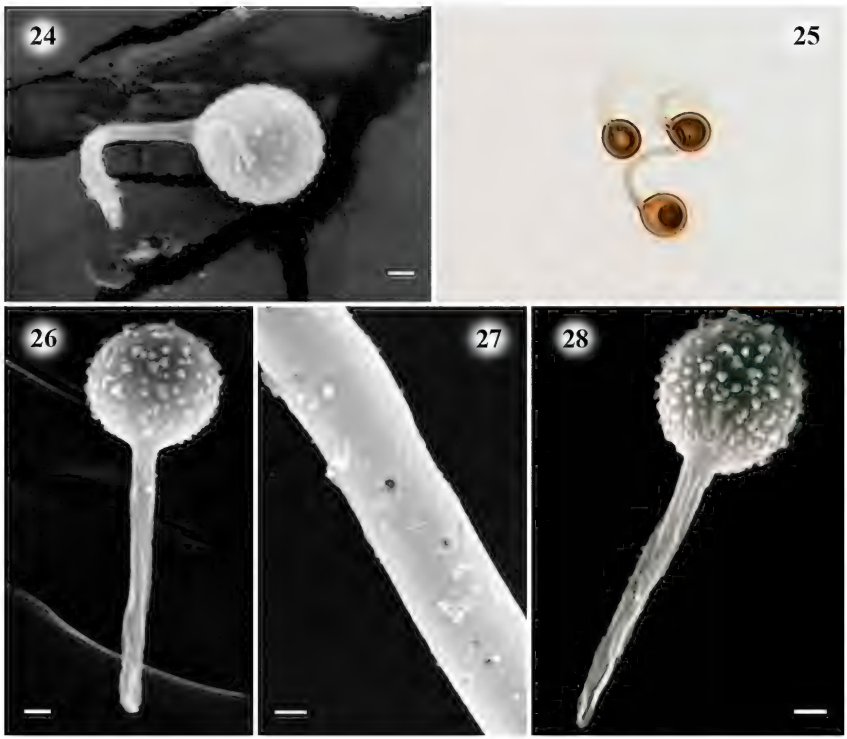
Observed in some of the examined specimens were clamp connections on brown dichotomously branching hyphae with thin walls and thin-walled septate ochraceous hyphae with projections shaped like small tubercles interspersed in the capillitium. Additionally, the pedicel surface appears smooth under the OM but rough and wrinkly with the SEM. It is notable that these details have not been reported in previous studies, although these are not diagnostic, with the exception of the pedicel, which is unique to *B. fusca.*

The taxonomy of *B. fusca* is complex and not very clear, because the material examined, particularly in the collections of young specimens, was easily confused with *Lycoperdon* basidiomes, given that they have a rigid granulose-squamulose peridium. As mentioned previously, *B. fusca* specimens are easily confused with other taxa precisely because our knowledge of this species is so scarce. Herrera (1959) described Mexican material as a new species, *B. ruizii*, which Kreisel (1967) synonymized with *B. fusca.*

The widely distributed *B. fusca* is very common in México's temperate forests and has been recorded for Aguascalientes (Pardavé 1991 [as *B. ruizii*]), Distrito Federal, Estado de México (Kreisel 1967), and Chihuahua (Moreno et al. 2010). This study extends its range to the states of Durango, Guerrero, Hidalgo, Jalisco, Michoacán, Morelos, Oaxaca, Puebla, Tlaxcala and Veracruz.

Bovista graveolens Schwalb, Lotos N. F. 13: 53, 1893. Figs 24–25

Specimens examined: **México. Estado de México:** La Marquesa-Tenango Highway, August 18, 1979 *E. Pérez-Silva* (MEXU 16052); Zinacantepec Municipality, km 17 Zultepec-Nevado de Toluca Highway near the town of Raíces, June 18, 1993 *A. Castellanos* (IZTA 2536). **Michoacán:** Zinapécuaro Municipality, Los Azufres long lagoon under forest protection, July 25, 1987 *Pompa-González 14* (FCME 13906). **Tlaxcala:** 2 km south of the La Malinche hostel, La Malinche Hill, July 19, 1987 *G. Mata 200* (XAL). **Veracruz:** La Joya, El Rodeo, February 18, 1985 *S. Chacón 3387 B* (XAL); Rafael Lucio Municipality, La Joya in front of the "La cabaña del chivo" restaurant, July 8, 1987 *F. Ramírez-Guillén 102* (XAL); Xico Municipality, eastern



Figs. 24–28. *Bovista graveolens*: 24. Spores under SEM. 25. Spores under OM ×1000. *Bovista leucoderma*: 26. Spores. 27. Pitted capillitium. *Bovista plumbea*: 28. Spore. Scale bars = 1 μm.

region of the Cofre de Perote mountain, Los Gallos, 15 km N of the El Rosario sugar refinery, May 15, 1995 *J. Rico 756* (XAL).

Comments — *Bovista graveolens* is characterized microscopically by the curved C-shaped pedicels on the majority of its spores that sometimes occur at the end of a widening corresponding to the basidium wall, although there are also spores with straight pedicels. The capillitium is very branched and the secondary hyphae are very long, compared with those of *B. fusca*. Although this species is easily confused with *B. fusca*, because its basidiomes are very similar, the differences become markedly evident under the microscope.

Bovista graveolens was known only for the state of Tlaxcala (Calonge et al. 2004b), but the present study broadens its distribution to include Estado de México, Michoacán, and Veracruz.

Bovista leucoderma Kreisel, Feddes Repert. 69: 203, 1964. Figs 26–27

Specimens examined: **México. Distrito Federal:** *S. González 97* (ENCB). **Hidalgo:** Loma Ancha, Marqués Dam, San Bartolo Socalpa, November 06, 1974

R. Cruz (ENCB); Omitlán de Juárez Municipality, Vicente Guerrero Ranch, ahead of the Real del Monte Mexico-Tampico Highway, short route km 115, July 05, 1986 *A. Calderón* et al. (MEXU 20142); Epazoyucan Peñas Largas Municipality, August 03, 1975 *M. Medina & I. García 1102* (ENCB); Zempoala Municipality, Tepa, November 20, 1976 *J. Baca del Moral* (ENCB); San Miguel Regla, August 16, 1980 *E. Pérez-Silva* (MEXU 17180). **Estado de México:** La Palma Hill, Salazar, October 20, 1957 *M. Ruiz-Oronoz* (MEXU 141); Between Chalco and Amecameca, September 05, 1965 *J. Álvarez* (MEXU 19864); km 39 Xochimilco-Oaxtepec Highway, August 24, 1980 *E. Fuentes* (MEXU 17098); San Pablo Teacalco, NE of Los Reyes, Mexico-Pachuca Highway, June 30, 1968 *J.L. Magaña 38-B* (ENCB); Tlamacas, foothills of Popocatépetl, August 31, 1958 *E. Pérez-Silva* (MEXU 889); Salazar, October 01, 1961 *Herrera, Zenteno & Riba* (MEXU 7805); October 19, 1969 *Herrera, Ulloa & Vázquez* (MEXU 6772); Purificación, NE of Texcoco, September 05, 1976 *G. Guzmán 16482* (ENCB); Tlalnepantla Municipality, Guadalupe Lake, September 15, 1968 *F. Martínez 61* (ENCB). **Puebla:** Tepeyahualco, May, 1999 *L. Mohedano 3* (XAL). **Veracruz:** Foothills of the Cofre de Perote mountain, October 30, 1982 *L. Guzmán-Dávalos 724* (ENCB).

Comments — *Bovista leucoderma* is mainly characterized by basidiomes with a white exoperidium that forms small plates in mature specimens. The *Bovista* type capillitium contains pores, pseudosepta and true septa.

This species has been cited for the states of Baja California (Ochoa 1993), Distrito Federal, Hidalgo, Puebla (Guzmán & Herrera 1973), and Estado de México (Kreisel 1967; Guzmán & Herrera 1973). Here it is reported for the first time for the state of Veracruz.

Bovista plumbea Pers., Ann. Bot. (Usteri) 15: 4, 1795. Fig 28

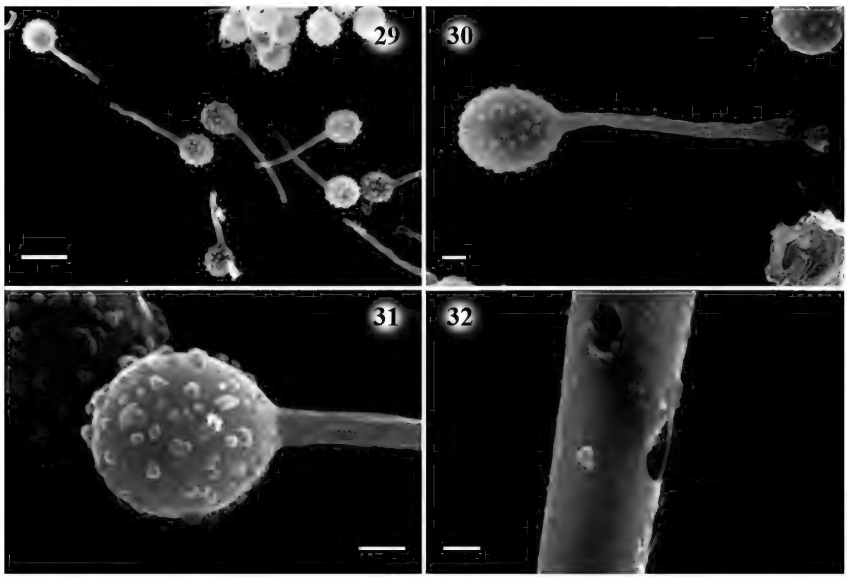
Specimens examined: **MÉXICO. Baja California:** Doña Petra Canyon, N of Ensenada, January, 1971 *S. Guzmán del Próo & S. de la Campa* (ENCB); El Junco, 15 km NE of Ensenada, February, 1983 *N. Ayala 46-B* (XAL); Vallecitos, San Telmo to Observatory of the UNAM road, San Pedro Mártir mountains, February 25, 1984 *G. Guzmán 24277-A* (XAL).

Comments — This species is easily recognized by its white exoperidium which, when removed, reveals the lead grey endoperidium.

In México, *B. plumbea* has been recorded only for Baja California (Ochoa & Moreno 1996, Ochoa et al. 2000); a previous report for Veracruz is rejected (Calonge et al. 2004b; Ochoa & Moreno 2006) as the cited specimen (L. Montoya-Bello 698 XAL) has been redetermined to *B. fusca*. Kreisel (1967) mentions that *B. plumbea* is distributed in northern New Zealand and over a vast region in Europe, Asia, and North America.

Bovista tomentosa (Vittad.) De Toni, Syll. Fung. 7: 97, 1888. Figs 29–32

Specimens examined: **MÉXICO. Baja California:** Turnoff at km 55 on the Ensenada-San Felipe Highway, km 15 road to the Hanson Lagoon, Juárez mountains, March 2, 1984 *Ayala 365*; *G. Guzmán 24328-A* (XAL). **Hidalgo:** Surroundings of San Gregorio, N side of Xihuingo Hill, July 26, 1963 *G. Guzmán 3875* (ENCB; MEXU 5548); Highway from Pachuca to Venados, turnoff to El Chico, Barranca Oriental, June



Figs. 29–32: *Bovista tomentosa:* 29–31. Spores. 32. Pitted capillitium. Scale bars: 29 = 5 µm; 30–32 = 1 µm.

24, 1973 *G. Guzmán 10874* (ENCB). **Estado de México:** Satélite City, June 29, 1982 *E. Pérez-Silva* (MEXU 22489); Zumpango Municipality, San Pablo Teacalco, June 30, 1968 *E. Quezada-Guzmán 10* (ENCB).

Comments — Specimens from the XAL herbarium identified as *B. minor* Morgan and *B. tomentosa* by Calonge et al. (2004b) were compared but no significant microscopic differences were found. Examination of an herbarium specimen from Belgium identified as *B. tomentosa* by Demoulin revealed that the XAL Mexican specimens cited above coincide both macro- and microscopically with the Belgian material.

Kreisel (1967) placed *B. tomentosa* (European) and *B. minor* (North American) in subg. *Globaria* sect. *Ovisporae* because of their ovoid spores and the *Bovista* type capillitium with pores. Microscopically, these two species are very similar, and Kreisel differentiated them based on their depth in the soil: *B. tomentosa* has one third of its basidiome immersed in the substrate and grows in arid dry open regions, while *B. minor* produces subhypogeous basidiomes with only the upper part above the soil and grows in humid regions in the shade. We believe that the relationship between basidiome and substrate is an ecological character lacking in taxonomic value. Because of the macro- and microscopic similarities between the two species, we suggest that they are conspecific, with *B. tomentosa* as the prior name.

Bovista tomentosa is characterized by the presence of mainly ovoid verrucose pedicellate spores and a *Bovista* type capillitium with pores. In some specimens both the primary and secondary hyphae have pores, while in others only the secondary hyphae have them.

Bovista tomentosa has been cited for the state of Baja California (Calonge et al. 2004b). This study extends its distribution range to Estado de México and Hidalgo.

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Subulicystidium curvisporum sp. nov. (*Hymenochaetales, Basidiomycota*) from the Patagonian Andes

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Abstract — *Subulicystidium curvisporum* is described as a new species from the Patagonian Andes forests. It differs from its closest related species, *S. longisporum* and *S. perlongisporum*, in considerably longer and characteristically curved basidiospores. A key to all known *Subulicystidium* species is included, and the new species is compared with other species in the genus.

Key words — Argentina, corticioid fungi, *Nothofagus dombeyi*, Patagonia, taxonomy

Introduction

The genus *Subulicystidium* Parmasto comprises 9 described species (Duhem & Michel 2001, Parmasto et al. 2004). *Subulicystidium* is an easily distinguished genus characterized above all by the distinctive cystidia encrusted with rectangular crystals arranged crosswise to the main axis ("two rows of "ribbon-like" structures," Jülich 1975), the presence of repetobasidia, and by the cylindrical to acicular basidiospores sharing a more or less vermiform shape. Molecular studies by Hibbett & Binder (2002) and Larsson et al. (2004) show that *Subulicystidium* is closely related to *Tubulicium vermiferum* (Bourdot) Oberw. ex Jülich, and both are included in the trechisporoid clade next to other *Trechispora* P. Karst. species, even if they do not share the same morphological and ecological characters.

As a result of collecting trips in the Patagonian Andes forests of southern Argentina, we have found growing on *Nothofagus dombeyi* (Mirb.) Oerst. (*Nothofagaceae*) a *Subulicystidium* species with long acicular and characteristically curved basidiospores, distinct from previously described species, which we propose as new below. Some species in *Tulasnella* J. Schröt. have spirally curved

basidiospores that often produce secondary spores by replication, but basidia are differentiated by the large globose to clavate sterigmata. We considered the possibility that a *Tulasnella* species inhabited the same substrata where the *Subulicystidium* grew, but we have not found any trace of a tulasnelloid fungus. *Tulasnella helicospora* Raunk., although somewhat inconspicuous, clearly differs in the grayish pruinose basidiome, more variably shaped wider basidiospores with homogeneous oily contents that germinate secondary spores, and typical basidia with obclavate distally tapered sterigmata.

Material & methods

For light microscopic studies, samples were mounted in 3% potassium hydroxide (KOH), Melzer's reagent (IKI), and 0.1% cotton blue in 60% lactic acid to determine cyanophily of basidiospores. The term Q expresses the ratio Length/Width of the basidiospore size. Line drawings were made with a camera lucida attachment. Specimens are deposited in the herbarium of the "Centro de Investigación y Extensión Forestal Andino-Patagónico" (CIEFAP, Esquel, Argentina), BAFC, and SALA.

Taxonomy

Subulicystidium curvisporum Gorjón, Gresl. & Rajchenb., **sp. nov.** PLATES 1,2

MYCOBANK MB 561691

Ab Subulicistidium longisporum *et* Subulicistidium perlongisporum *differt basidiosporae longioribus et recurvis.*

HOLOTYPUS: Argentina, Chubut, Los Alerces National Park, Alerzal milenario (42°36′34″S 71°53′28″W), 530 m a.s.l., on decayed wood of *Nothofagus dombeyi*, 3 May 2010, leg. S.P.Gorjón coll. 2689. **Holotype**, BAFC; **isotypes**, SALA and herbarium of Centro de Investigación y Extensión Forestal Andino-Patagónico.

ETYMOLOGY: *curvisporum,* Latin, referring to the curved shape of the basidiospores.

BASIDIOMATA annual, fragile, resupinate, effused, arachnoid, smooth and finely velutinous due to the protruding cystidia, white to light grey, margin abrupt. HYPHAL SYSTEM monomitic, generative hyphae with clamps, thin- to thick-walled, 3–4 µm wide, smooth or encrusted with amorphous hyaline material. CYSTIDIA numerous, subulate, 70–80(–100+) × 3–4 µm, projecting 60–80 µm above the basidia, encrusted over all the length, except in the smooth apical part, with rectangular crystals circularly to spirally arranged along the cystidial wall. BASIDIA urniform, basally clamped, some of them repetitive, thin-walled, 12–15 × 5–7 µm, with 4 stout sterigmata. BASIDIOSPORES long acicular, spirally curved, with a vermiform shape, smooth, thin-walled, (25–)27–35(–38) × (1.8–)2–2.5(–2.8) µm, inamyloid, nondextrinoid, acyanophilous, guttulate (Plates 1, 2).

DISTRIBUTION AND ECOLOGY —*Subulicystidium curvisporum* is only known from the Patagonian Andes forest of Argentina (Valdivian rainforest).

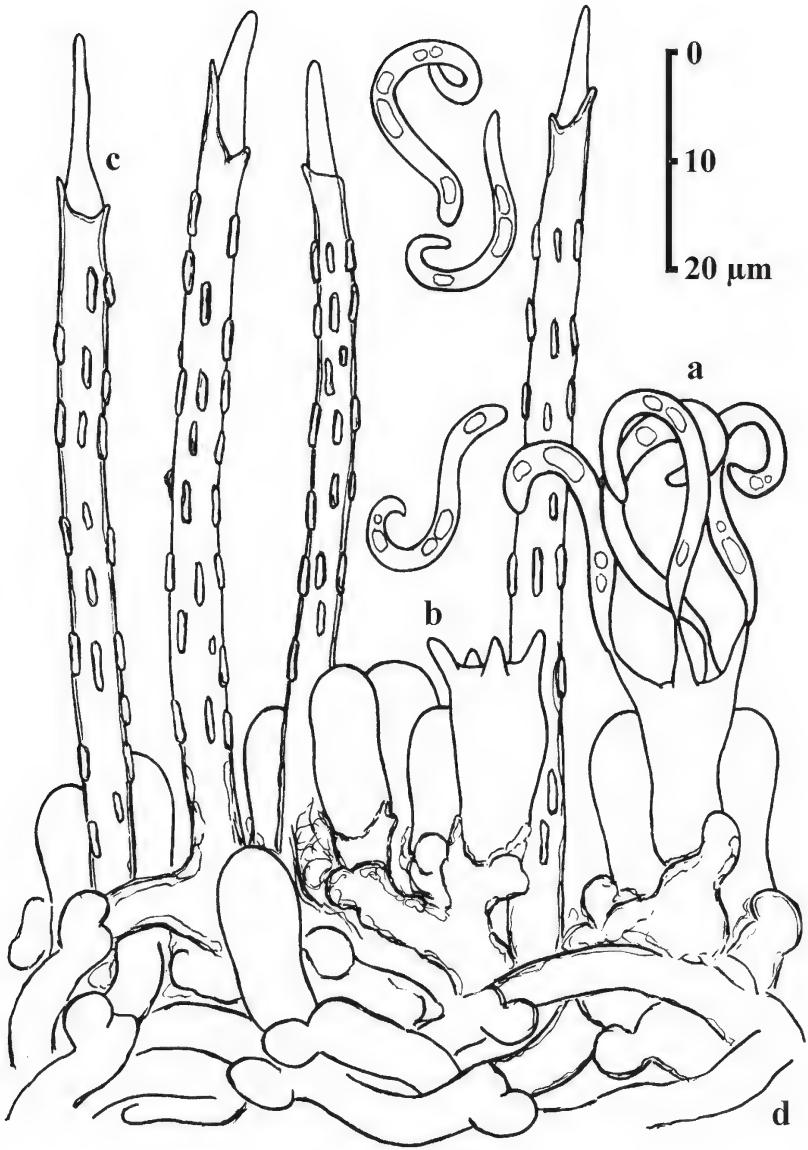

Plate 1. *Subulicystidium curvisporum*.
Hymenial elements— a) basidiospores, b) basidia, c) cystidia, d) generative hyphae.
coll. S.P.Gorjón 2689, holotype.

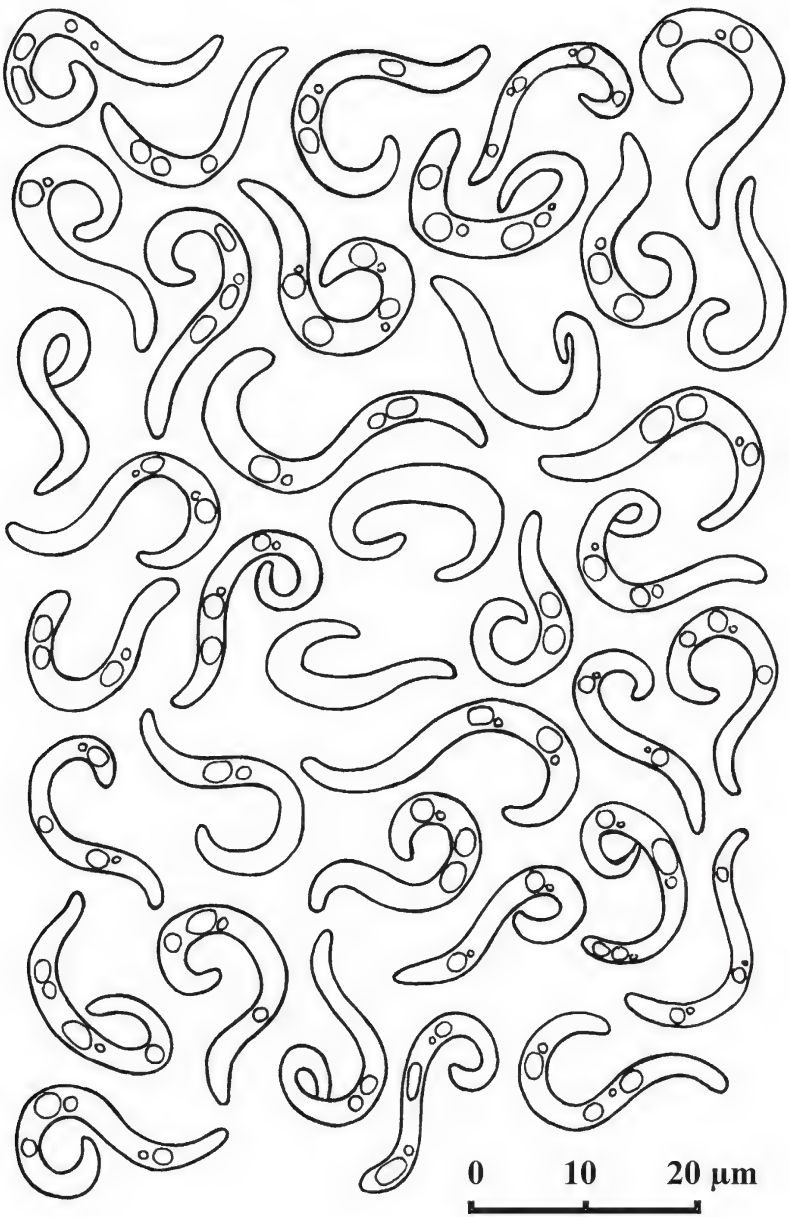

Plate 2. *Subulicystidium curvisporum*. Basidiospores, coll. S.P.Gorjón 2689, holotype.

It grows on decayed wood of *Nothofagus dombeyi*, an endemic southern South American perennial hardwood, in cold rainforests mixed with *Fitzroya cupressoides* (Molina) I.M. Johnst. (*Cupressaceae*), *Luma apiculata* (DC.) Burret (*Myrtaceae*), and *Chusquea culeou* E. Desv. (*Poaceae*).

Additional specimens examined – ***Subulicystidium curvisporum***. **ARGENTINA. Chubut:** Los Alerces National Park, Alerzal milenario, on decayed wood of *N. dombeyi*, 3 Mar 2011, leg. S.P.Gorjón, *SPG 3023*.

Subulicystidium longisporum. **ARGENTINA. Tierra del fuego:** Depto. Ushuaia, Estancia El Valdez, on dead wood of *Nothofagus betuloides* and *N. pumilio*, 4 Mar 1996, leg. A. Greslebin, *AG 340, 382*; Monte El Martial, on *N. pumilio*, 28 Mar 1998, leg. M. Rajchenberg, *MR 11564*. **CHILE. X Region:** Puyehue, Entre Lagos, close to Gol-Gol river bridge, on decayed wood of *N. dombeyi*, 21 Feb 2010, leg. N. Hallenberg & S.P. Gorjón, *SPG 2600*.

Tulasnella helicospora. **ARGENTINA. Río negro:** Mallín Ahogado, El Guadal, on bark of fallen trunk of *Austrocedrus chilensis* (D. Don) Pic.Serm. & Bizarri (*Cupressaceae*), 5 Sep 1995, leg. M. Rajchenberg, *MR 11015*.

Comments — *Subulicystidium curvisporum* belongs to the group of *Subulicystidium* species with long filiform to acicular basidiospores with a Q > 4. Among them, *Subulicystidium longisporum* (Pat.) Parmasto, a common globally distributed species, has the shortest basidiospores with a Q = 4–7. The closest related species seems to be *Subulicystidium perlongisporum* Boidin & Gilles (known from Réunion Island, Tropical Africa, Japan, France, and the Caucasus) with smaller and straight basidiospores (Boidin & Gilles 1988). *Subulicystidium cochleum* Punugu, known from St. Lucia in the Lesser Antilles, has smaller basidiospores with the distal end slightly folded back or twisted and curved; *S. cochleum* differs also in the cystidial ornamentation composed of long acicular crystals arranged in a cylinder and restricted to the middle part (Punugu et al. 1980). The remaining species —*S. allantosporum* Boidin & Gilles, *S. brachysporum* (P.H.B. Talbot & V.C. Green) Jülich, *S. meridense* Oberw., *S. naviculatum* Oberw., *S. nikau* (G. Cunn.) Jülich, *S. obtusisporum* Duhem & H. Michel— have differently shaped basidiospores, usually reniform or cylindrical to fusiform and with a Q<4. Limits between some *Subulicystidium* species are unclear, and spore size frequently overlaps. As Liberta (1980) already stated, it is likely that some species belong to a single species complex.

Key to *Subulicystidium*

1a. Basidiospores acicular, Q >4 2
1b. Basidiospores fusiform, cylindrical to reniform, Q <4 5
2a. Basidiospores (9–)12–16(–18) × (1.8–)2–3 µm, Q = 4–7 ..... ***S. longisporum***
2b. Basidiospores longer, Q >9 3
3a. Basidiospores spirally curved, (25–)27–35(–38) µm long ..... ***S. curvisporum***
3b. Basidiospores straight or only slightly curved, shorter 4

4a. Basidiospores 20–27 × 2–3 µm, cystidial encrustation restricted to the middle part of the cystidium ..... ***S. cochleum***

4b. Basidiospores 16–25 × 1.5–2.3 µm, cystidia encrusted over the entire length ..... ***S. perlongisporum***

5a. Basidiospores 4–5 µm wide 6

5b. Basidiospores up to 4 µm wide 7

6a. Basidiospores navicular, 10–12 × 4.5–5 µm ..... ***S. naviculatum***

6b. Basidiospores phaseoliform, 7–9.2 × 4–5 µm ..... ***S. nikau***

7a. Basidiospores (2.5–)3–4 µm wide 8

7b. Basidiospores 2–2.5(–3) µm wide 9

8a. Basidiospores shorth cylindrical, 6–8 × 3–3.6 µm ..... ***S. allantosporum***

8b. Basidiospores cylindrical to fusoid, slightly curved, (9–)11–13(–15) × (2.5–)3–4 µm ..... ***S. obtusisporum***

9a. Basidiospores cylindrical, 6.5–8.5 × 2.2–2.5 µm ..... ***S. meridense***

9b. Basidiospores cylindrical to fusiform or banana-shape, (6.5–)7.5–10 × 2–2.5(–3) µm ..... ***S. brachysporum***

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New records of smut fungi. 4. *Microbotryum coronariae* comb. nov.

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Abstract — For *Ustilago coronariae* on *Lychnis flos-cuculi,* a new combination in *Microbotryum, M. coronariae,* is proposed. It is reported as new to Bulgaria.

Key words — *Microbotryaceae,* taxonomy

Introduction

A new combination in *Microbotryum, M. coronariae,* is proposed for *Ustilago coronariae* on *Lychnis flos-cuculi.*

Material & methods

Dried specimens from SOMF and (on loan) BP, BPI, COLO, DAOM, FH, H, ILL, ILLS, ISC, KRAM, M, KSC, MICH, NY, and W were examined under light (LM) and scanning electron (SEM) microscopes. For LM observations, spores were mounted in lactophenol solution on glass slides, gently heated to boiling point to rehydrate the spores, and then cooled. Spore measurements are given in the form: min–max (extreme values, if necessary) [mean ± 1 standard deviation]. The total number of spores (n) from all collections (x) measured are given in the form '(n/x)'. For SEM, spores were attached to specimen holders by double-sided adhesive tape and coated with gold with an ion sputter. The surface structure of spores was observed at 10 kV and photographed with a JEOL SM-6390 scanning electron microscope.

Taxonomy

Liro (1924) described *Ustilago coronariae* as a species morphologically identical with *U. dianthorum* Liro, *U. lychnidis-dioicae* Liro, and *U. stellariae* (Sowerby) Liro but with a narrow specialization on *Coronaria flos-cuculi* (L.) A. Braun (currently *Lychnis flos-cuculi*). Using artificial infections with *Ustilago coronariae,* Liro (1924: 319) demonstrated that the following caryophyllaceous plants were not hosts: *Agrostemma githago* L., *Dianthus deltoides* L., *Lychnis*

chalcedonica L., *Silene dioica* (L.) Clairv. (as *Melandrium rubrum*), *Silene latifolia* subsp. *alba* (Mill.) Greuter & Burdet (as *Melandrium album*), *Silene nutans* L., *Silene vulgaris* (Moench) Garcke (as *S. inflata*), *Stellaria graminea* L., *Stellaria holostea* L., and *Viscaria vulgaris* Bernh. Later, *Ustilago coronariae* was reduced to a synonym of *Ustilago violacea* and, after its transfer to *Microbotryum,* to *M. violaceum* s. lat. (cfr Vánky 1994: 156). Based on physiological (Liro 1924) and molecular phylogenetic (Le Gac et al. 2007, Refrégier et al. 2008, Devier et al. 2010) inferences, we propose that *Ustilago coronariae* be transferred to *Microbotryum* as an independent species.

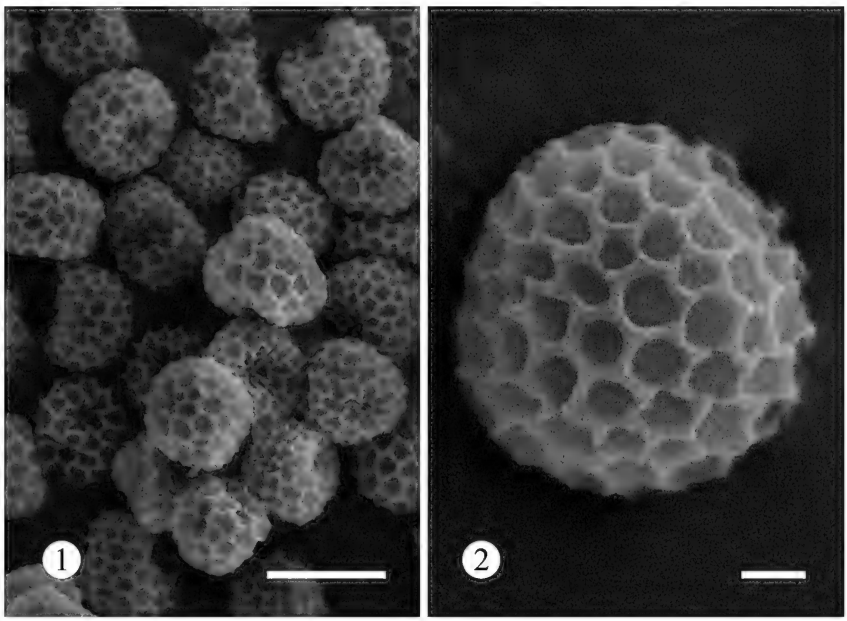
Microbotryum coronariae (Liro) Denchev & T. Denchev, **comb. nov.** FIGS 1–2

MycoBank MB 561573

≡ *Ustilago coronariae* Liro, Annales Academiae Scientiarum Fennicae, Ser. A 17(1): 38, 1924. — Lectotype on *Coronaria flos-cuculi* (design. by Lindeberg 1959: 142), Finland, Regio aboënsis, Turku [Åbo], 11 July 1916, J.I. Liro & E. Kitunen; isolectotypes in Liro, Mycoth. fennica, no. 347.

SORI in anthers. SPORE MASS powdery, dark livid, dark purple or dark vinaceous (based on the Rayner (1970) colour chart). SPORES mainly globose or subglobose, sometimes broadly ellipsoidal or ovoid, 6.5–10(–11) × 5.5–9.5 [7.7 ± 0.7 × 7.2 ± 0.6] µm ($n/_{19}$=1750); spore wall reticulate, 6–8 meshes per spore diameter, meshes irregularly polygonal, (0.5–)0.7–1.4(–1.6) µm long; in SEM interspaces smooth.

SPECIMENS EXAMINED — On *Lychnis flos-cuculi* L.: **BULGARIA,** Mt. Vitosha, near Chouypetlyovo, 1250 m, 24 July 1992, C.M. Denchev (SOMF 29192); **ESTONIA,** island Saaremaa, 1899, T. Vestergren (as *U. violacea,* DAOM 215227); **FAEROE ISLANDS,** Kalsö, 5 August 1897, J. Hartz & C. Ostenfeld (as *U. coronariae,* H); **FINLAND,** Ab, **isolectotype,** Mycoth. fennica, no. 347 (H); Ab, Turku, Katariinan laakso, 11 July 1916, E. Kitunen & J.I. Liro (as *U. coronariae,* H); N, Vantaa, Tikkurila, 21 June 1919, A. Rainio (as *U. violacea,* H); Satakunta, Kankaanpää, Kunintaanlähde, 8 August 1935, M. Laurila (as *U. coronariae,* H); Satakunta, Yläne, Elijärvi, Vastalahti, 60°54' N, 17 July 1950, L.E. Kari (as *U. violacea,* Fungi exs. fennici, no. 710, MICH, NY, W 8496); Tb, Jyväskylä, Vuoritsalo, 7 July 1916, K. Linkola (as *U. coronariae,* H); **FRANCE,** 30 May 1880, N. Patouillard (as *U. antherarum,* FH); **GERMANY,** Bayern, Bez. Kelheim, Ulrain, 12 June 1940, E. Eichhorn (as *U. coronariae,* M); Nordrhein-Westfalen, Kr. Siegen, Freudenberg, 17 June 1934, A. Ludwig (as *U. coronariae,* BPI 159712, FH); Brandenburg, Berlin, Rahnsdorf, 16 June 1935, Fahrendorff (as *U. coronariae,* M, W 8999); Genshagen s. Berlin, 23 June 1899, P. Sydow (as *U. violacea,* Sydow, Ustilag., no. 214, FH, KSC, M, NY); Mellen bei Zossen, 9 June 1905, P. Sydow (as *U. violacea,* Sydow, Mycoth. german., no. 367, COLO F-6367, FH 1094144, ILL 17344, M, MICH; the host plant wrongly given as *Viscaria vulgaris*); Kr. Teltow, Trebbin, April 1946, W. Lempke (as *U. coronariae,* BPI 159710); **LATVIA,** prov. Vidzeme, Triedaine, 16 July 1932, A. Kirulis (as *U. coronariae,* BPI 159709, ILLS 23414); **NETHERLANDS,** Wageningen, 26 June 1923, J.I. Liro (as *U. violacea,* H); **POLAND,** Łęknica (on the label as "Muskau, Lugknitz"; cfr Scholz & Scholz 1988: 231), June 1891, P. Sydow (Sydow, Mycoth. march., no. 3222) (as *U. violacea,* BP 3033, NY); Dąbroszyn (formerly German Tamsel), 28 June 1925, P. Vogel (as *U. coronariae,* Sydow, Mycoth. german., no. 2290, BPI 159708, COLO F-8290, FH 1095112, ISC 371037, MICH, W 922); "Warthewiesen bei Tamsel", 28 June 1925, P. Vogel (as *U. violacea* f. sp. *lychnidis-*



Figs 1–2. Spores of *Microbotryum coronariae* on *Lychnis flos-cuculi* in SEM (Isolectotype, H). Scale bars: 1 = 10 µm; 2 = 1 µm.

flos-cuculi, Zillig, Ustilag. Europ., no. 59, M, W 10982); ditto, June 1926, P. Vogel (as *U. coronariae,* Petrak, Mycoth. gener., no. 1077, BPI 159713); Kórnik prope Poznan, June 1927, A. Wróblewski (Wróblewski et Siemaszko, Fungi polon. selec. exs., no. 7, KRAM-F 2964); **Russia,** Karelia, Kpocc, Iso-Keilak, 1 August 1896, Bergroth & J.I. Liro (as *U. coronariae,* H); Karelia, Kpor, Jernema – Somba, 17 August 1899, J.I. Liro (as *U. coronariae,* H); Karelia, Kpor, Prilug – Siftuga, 12 August 1899, J.I. Liro (as *U. coronariae,* H); Karelia, Kpor, Tamitsa, 26 July 1899, J.I. Liro (as *U. coronariae,* H); Karelia, Kl, Kankala, ad rivulum lacuum Valkialampi et Parijärui, 1 August 1935, M. Laurila (as *U. coronariae,* H); Sieverskaja, prov. Petropolitanae, 6/18 June 1898, Tranzschel (Jaczewski, Komarov & Tranzschel, Fungi rossiae exs., no. 207) (as *U. violacea,* FH, NY); Mikhaylovskoe, Moskow Gubernia, 28 June 1917, F. Bucholtz (as *U. violacea,* FH, NY); **Sweden,** prope Stockholm, 20 June 1883, E. Eriksson (as *U. violacea,* Eriksson, Fungi paras. scand. exs., no. 153a, NY); Östergötland, Gryt parish, Korsudden, 16 July 1957, J.A. Nannfeldt, no. 14874 (as *U. violacea,* Fungi exs. suecici, no. 3017, W 13061); Öl, Stara Röv., June 1900, G. Lagerheim (as *U. violacea,* FH, H); Ramsåsa, 28 June 1929, H. Christoffersson (as *U. coronariae,* H); **Switzerland,** Canton de Vand, Emrions de Leysiu, 11 July 1917, E. Mayor (as *Ustilago coronariae,* BPI 159 711).

Distribution — On *Caryophyllaceae: Lychnis flos-cuculi,* Europe (Austria, Bulgaria, Czech Republic, Estonia, the Faeroes, Finland, France, Germany, Latvia, Lithuania, the Netherlands, Poland, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, UK) (Liro 1924; Săvulescu 1957; Lindeberg 1959; Ignatavičiūtė 1975, 2001; Mordue & Ainsworth 1984; Ványky 1985; Zogg 1986; Scholz & Scholz 1988; Karatygin & Azbukina 1989; Almaraz 2002; Zwetko & Blanz 2004; Legon et al. 2005; etc.). The smut is reported here as new to Bulgaria.

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Lentinus giganteus revisited: new collections from Sri Lanka and Thailand

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Abstract— A new collection of *Lentinus giganteus* from Sri Lanka, where it was originally described, is used to epitypify the species after comparison with the type protologue and drawings held in Peradeniya, Sri Lanka; a full description and illustrations are provided. Additional collections were made at three sites in northern Thailand. Phylogenetic ITS-1-5.8S-ITS2 rDNA sequence analyses using maximum likelihood, maximum parsimony and Bayesian inference all support the transfer of *L. giganteus* to *Pleurotus*. Although the collections from Thailand differ slightly morphologically and phylogenetically from *P. giganteus* sensu stricto, these differences do not yet merit specific status. Instead, *P. giganteus* is maintained as one widely variable species represented by relatively large fruiting bodies. Saprobic on buried well-rotted wood in forests, *P. giganteus* is widely consumed in Sri Lanka and might be profitably cultivated in Thailand.

Key words— edible fungi, morphology, new records, new combination, taxonomy

Introduction

Research on macrofungi of the Mushroom Research Centre and surrounding areas in northern Thailand has documented a remarkable biodiversity and produced many new species and new records (Le et al. 2007a,b; Sanmee et al. 2008; Kerekes & Desjardin 2009; Wannathes et al. 2009a,b; Zhao et al. 2010). Recently we have focused on potentially cultivatable and edible genera (Karunarathna et al. 2011). This paper presents the first report of *Lentinus giganteus* from Thailand.

Lentinus Fr. is a cosmopolitan genus with an estimated 40 species (Kirk et al. 2008) distributed across a wide temperature range, being abundant in the tropics and often found in temperate regions (Pegler 1983). *Lentinus* species, normally wood decaying basidiomycetes, are characterized by decurrent lamellae, dimitic tissues, and hyaline ellipsoid to cylindrical basidiospores. Species in subgenus *Lentinus* have hyphal pegs (Corner 1981, Pegler 1983). Generally the xeromorphic long-lived basidiomes are tough and firm when dry, but in Thailand they fruit only at the beginning of the rainy season (Sysouphanthong et al. 2010, Karunarathna et al. 2011). Traditionally, *Lentinus* was placed in the agaric family *Tricholomataceae* based on the lamellate hymenophore and white spore print (e.g., Miller 1973).

Previous researchers (e.g., Redhead & Ginns 1985; Hibbett & Vilgalys 1991, 1993; Hibbett & Thorn 1994; Hibbett et al. 1993) noted the existence of a *Lentinus–Pleurotus–Panus* complex and showed that *Lentinus* sensu Pegler is polyphyletic, with the monophyletic *Lentinus* subg. *Lentinus* sensu Pegler belonging to the *Polyporales* (Hibbett 1991, Kruger & Gargas 2004). *Panus* Fr., *Pleurotus* (Fr.) P. Kumm., and *Neolentinus* Redhead & Ginns are also monophyletic, with *Pleurotus* belonging to the *Agaricales* (Fleming 1994, Hibbett et al. 1994). Corner (1981), Kühner (1980), Pegler (1975, 1983) and Singer (1986) all classified these genera differently.

Lentinus giganteus, originally described from Sri Lanka (Berkeley 1847), bears many structures that are atypical of *Lentinus*, and its taxonomic position has long been unresolved (Pegler 1983). Corner (1981: 54) suggested it as type (and only species) of his new subgenus, *Panus* subg. *Gigantopanus* Corner. Pegler (1983: 168) transferred this subgenus (as a section) to *Lentinus: L.* sect. *Gigantopanus* (Corner) Pegler. Even though the largest spores become oblong-ellipsoid, mature spores are not cylindrical but somewhat broadly ellipsoid. The oil guttule present inside the spore is characteristic of *Lentinus*. The generative hyphae are narrower than the skeletal hyphae. The lamellar edge is distinct with a broad, sterile layer of lecythiform cheilocystidia similar to those observed in some *Pleurotus* species. The lamellae are wide and well separated, and the development process is metavelangiocarpic. In many ways this species might be more properly positioned in *Pleurotus*, rather than in *Lentinus*. However,

the skeletal hyphae of the dimitic hyphal system dominate to produce a tough basidiome and the radiate construction of the hymenophoral trama differentiates with the descending trama observed in *Pleurotus*.

Lentinus giganteus, referred to as "uru paha" in Sri Lanka, is one of the largest edible mushrooms and —as noted in Buddhist literature— has been treated as a special food since ancient times (Udugama & Wickramaratna 1991; Berkeley 1847).

Objectives of the present study were to redescribe *L. giganteus* from fresh collections from the type locality, epitypify *L. giganteus* with a fresh Sri Lankan collection, decide on the taxonomic position of *L. giganteus* to determine whether it is related to *Lentinus* or *Pleurotus* using ITS-1-5.8 S-ITS2 rDNA sequence data, and introduce the species to Thailand as a new edible mushroom.

Materials & methods

Lentinus giganteus samples were collected in northern Thailand and Sri Lanka between June 2008 and October 2010 and processed as in Karunarathna et al. (2011).

Ethnomycological survey

Thirty locals including mushroom sellers in markets were surveyed through a general questionnaire in Chiang Mai and Chiang Rai provinces, Thailand.

Morphological examination

Macro-morphological characters were described from fresh material and documented by photographs. Colour designations (e.g., 4B5) follow Kornerup & Wanscher (1978), while the colour names (e.g., grayish yellow) follow Ridgway (1912). Specimens were dried, placed in separate plastic bags, and deposited in the Herbarium of Mae Fah Luang University (MFLU). For microscopical examination, sections were cut with a razor blade from dried specimens, mounted on slides in 5% KOH and Congo red, observed, measured, and illustrated using a Zeiss Axioskop 40 compound microscope. Basidiospore measurement abbreviations: n = number of spores measured; L_m = mean spore length; W_m = mean spore width; Q = length/width ratio (L/W) of a spore in side view; Q_m = average Q of all spores measured.

Molecular & phylogenetic methods

DNA EXTRACTION— Genomic DNA was extracted from dried mushroom samples with cetyl trimethylammonium bromide (CTAB) according to Doyle & Doyle (1990) with some modifications. DNA concentrations were estimated visually in agarose gel by comparing band intensity with a 1000 bp DNA ladder (Transgen Biotech).

PCR AMPLIFICATION & SEQUENCING—PCR reactions were performed in a 50 µl volume (0.625 mM primers (White et al. 1990: ITS4: 5′TCCTCCGCTTATTGATATGC3′; ITS5: 5'GGAAGTAAAAGTCGTAACAAGG3′); 10–20 ng DNA template, 10× buffer, 0.2 mM dNTPs, 1.5 units Taq and sterile water. Thermal cycles: 3 m at 94 °C, 30–35 cycles at 93 °C for 30 s, 1 m at 55 °C, 1 m at 72 °C, final extension 10 m at 72 °C. PCR products were verified by 1% agarose electrophoresis gels stained with ethidium bromide in 1×Tris-boric acid EDTA buffer. ITS 4/ITS 5 were used to sequence both DNA strands (White et al. 1990) at the National Botanic Gardens of Belgium.

Table 1. *Pleurotus giganteus* and other taxa sequenced for phylogenetic analyses.

Taxa	Country of origin	GenBank accession numbers (ITS)
Lentinus squarrosulus	Japan	AB478883 (Sotome et al. 2009)
Panus sp.	China	HM245784 (unpublished)
P. australis	New Zealand	AY315764 (Zervakis et al. 2004)
P. cornucopiae	Austria	AY450341 (unpublished)
P. cystidiosus	India	AY315810 (Zervakis et al. 2004)
P. euosmus	China	EU424298 (unpublished)
P. giganteus (MFLU 08 1370)	Thailand	(new sequence, from dried sample)
P. giganteus (MFLU 08 1371)	Thailand	(new sequence, from dried sample)
P. giganteus (MFLU10 0141)	Thailand	(new sequence, from dried sample)
P. giganteus (MFLU 11 0018, epitype)	Sri Lanka	(new sequence, from dried sample)
Pleurotus giganteus [as *Lentinus giganteus*]	Thailand	DQ334857 (unpublished)
P. giganteus [as *Panus giganteus*]	China	HM245788 (unpublished)
P. giganteus [as *Panus giganteus*]	China	HM245786 (unpublished)
P. giganteus [as *Panus giganteus*]	China	HM245780 (unpublished)
P. fuscosquamulosus	India	AY315789 (Zervakis et al. 2004)
P. smithii	Mexico	AY315779 (Zervakis et al. 2004)

Sequence alignment & phylogenetic analysis—Taxa sequenced and GenBank accession numbers are listed in Table 1. Sequences for each strain were aligned using Clustal X (Thompson et al. 1997). Alignments were manually adjusted to allow maximum sequence similarity. Gaps were treated as missing data. Phylogenetic analysis were performed using PAUP* 4.0b10 (Swofford 1998). Ambiguously aligned regions were excluded from all analyses. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Maxtrees were unlimited, branches of zero length were collapsed and all multiple parsimonious trees were saved. Clade stability of the trees resulting from the parsimony analyses were assessed by bootstrap analysis with 1000 replicates, each with 10 replicates of random stepwise addition of taxa (Felsenstein 1985). Trees were figured in TreeView.

Results

The ITS sequence dataset comprised 16 sequences (Table 1) representing three *Panus giganteus* collections and one *Lentinus giganteus* collection from GenBank, three *L. giganteus* collections from northern Thailand, and one collection from Sri Lanka. Outgroup taxa were *Lentinus squarrosulus* and *Panus* sp. Of 732 total characters, 363 are constant, 112 are parsimony-uninformative, and 257 are parsimony-informative. The MP tree was produced after 238,115

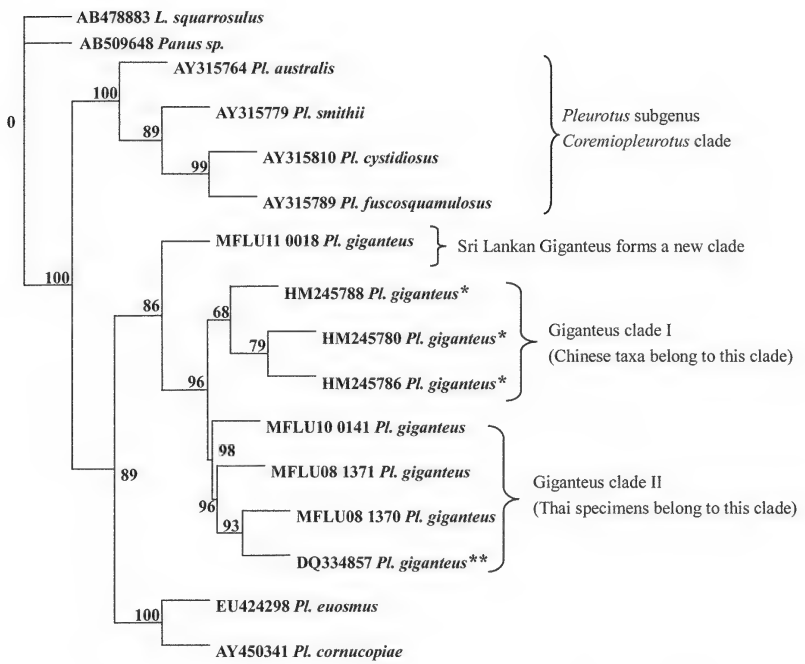

Plate 1. Maximum parsimony phylogram showing phylogenetic relationships among *Pleurotus giganteus* from Thailand (MFLU08 1370, MFLU08 1371, MFLU10 0141) and epitype from Sri Lanka (MFLU11 0018) with some selected *Lentinus*, *Panus* and *Pleurotus* species based on ITS-1-5.8 S-ITS2 rDNA sequences. Data were analysed with random addition sequence, unweighted parsimony and gaps were treated as missing data. Values above the branches are parsimony bootstrap (≥ 50%). The tree is rooted with *Lentinus squarrosulus* (AB478883) and *Panus* sp. (AB509648). *Pl* = *Pleurotus*; ** = as *Lentinus giganteus* in GenBank, * = as *Panus giganteus* in GenBank.

rearrangements; the best MP tree found (scored at 645) was chosen to represent the phylogenetic position of *L. giganteus* (Plate 1).

The three *L. giganteus* samples from Thailand (MFLU08 1370, MFLU08 1371, MFLU10 0141) and Chinese collections from GenBank form one clade (Plate 1) that is sister (86% bootstrap support) to the only *L. giganteus* collection from Sri Lanka. Both Sri Lankan and Thai collections are closely related to *Pleurotus cornucopiae* and *P. euosmus* (89% bootstrap support). The *L. giganteus* clade, *P. cornucopiae,* and *P. euosmus* cluster with *Pleurotus* subg. *Coremiopleurotus* (100% bootstrap support). *Coremiopleurotus* comprises *P. australis, P. smithii, P. cystidiosus,* and *P. fuscosquamulosus.*

These results support the view that *L. giganteus* is related to *Pleurotus* and not *Panus* or *Lentinus.* Since the morphological data also supports the molecular

conclusions, we transfer *L. giganteus* to *Pleurotus* and epitypify the species based on the collection from the holotype locality to enable future molecular studies.

Taxonomy

Pleurotus giganteus (Berk.) Karunarathna & K.D. Hyde, **comb. nov.** PLATES 2–4

MYCOBANK MB 561087

≡ *Lentinus giganteus* Berk., Lond. Journ. Bot. 6: 493[bis], pl.17/18 f.2 (1847)

≡ *Pocillaria gigantea* (Berk.) Kuntze, Revis. Gen. Pl. 2: 866 (1891)

≡ *Velolentinus giganteus* (Berk.) Overeem, Bull. Jard. Bot. Buitenz, 3 sér., 9: 12 (1927)

≡ *Panus giganteus* (Berk.) Corner, Beih. Nova Hedwigia 69: 69 (1981)

TYPE: Sri Lanka, Central Prov., Hautane Range, on ground, July 1844, by Gardner, No 58 (holotype K; PLATE 2A-C); Central Prov., Kandy Distr., Deliwala village, 7°14′43.55″N 80°33′51.40″E, elevation 1050 m, rainforest dominated by *Swietenia* spp. and *Artocarpus heterophyllus*, 5 June 2009 (MFLU11 0018, **epitype designated here** [PLATE 4A]).

PILEUS 60–310 mm in diameter, strongly convex to applanate becoming slightly depressed in the centre, dark brown (7F5), towards the margin light brown camel (6D4), grayish orange (5B4) at the marginal area, at centre fibrillose-scaly, surface initially uniformly dark, fuscous brown, fuliginous or black, then fading with age to pale ochraceous or yellowish brown (E8), with a darker centre although sometimes remaining dark, dry, distrupted into small, indefinite, radial, innate squamules, overlain by scanty, pale grey or blackish, verrucose-floccose, concentrically arrange remnants of the veil; margin strongly involute then straight, thin, slightly sulcate-striate. LAMELLAE moderately crowded with lamellulae of five lengths, decurrent, slightly interveined and anastomosing over the stipe apex, 2–3 mm broad, white to cream (3A2); edge entire, pale ochraceous or yellowish brown (E8). STIPE up to 50–200 mm long, 7–10 mm broad at the apex, 10–15 mm at the base, fusiform, with radicating base, solid, with surface concolorous with the pileus, paler at the apex, finely tomentose with indefinite zones of paler velar remnants in the early stages; veil thin, floccose, pale to dark brown (6F6), soon reduced to floccose remnants but never forming an annulus on the stipe. CONTEXT 5–10 mm thick at the disk, submembranous at the pileal margin, white in pileus and stipe, fleshy-spongy, consisting of a dimitic hyphal system with skeletal hyphae.

GENERATIVE HYPHAE (PLATE 3e) 4–6 µm in diameter, inflating with a thick or slightly thickened wall, more or less radially parallel but frequently branching and with large clamp connections. SKELETAL HYPHAE (PLATE 3d) 6–8 µm in diameter, hyaline of intercalary or terminal origin, becoming very thick-walled with a narrow lumen, tending to taper apically, occasionally with a limited lateral branch. BASIDIOSPORES (PLATE 3a) 7–9 × 6–7 µm [n = 40, L_m= 8.30 µm, W_m= 6.36 µm, Q= 1.18–1.46, Q_m= 1.33] broadly ellipsoid to ellipsoid, white in mass, smooth, with one large oil drop or multiguttulate, inamyloid,

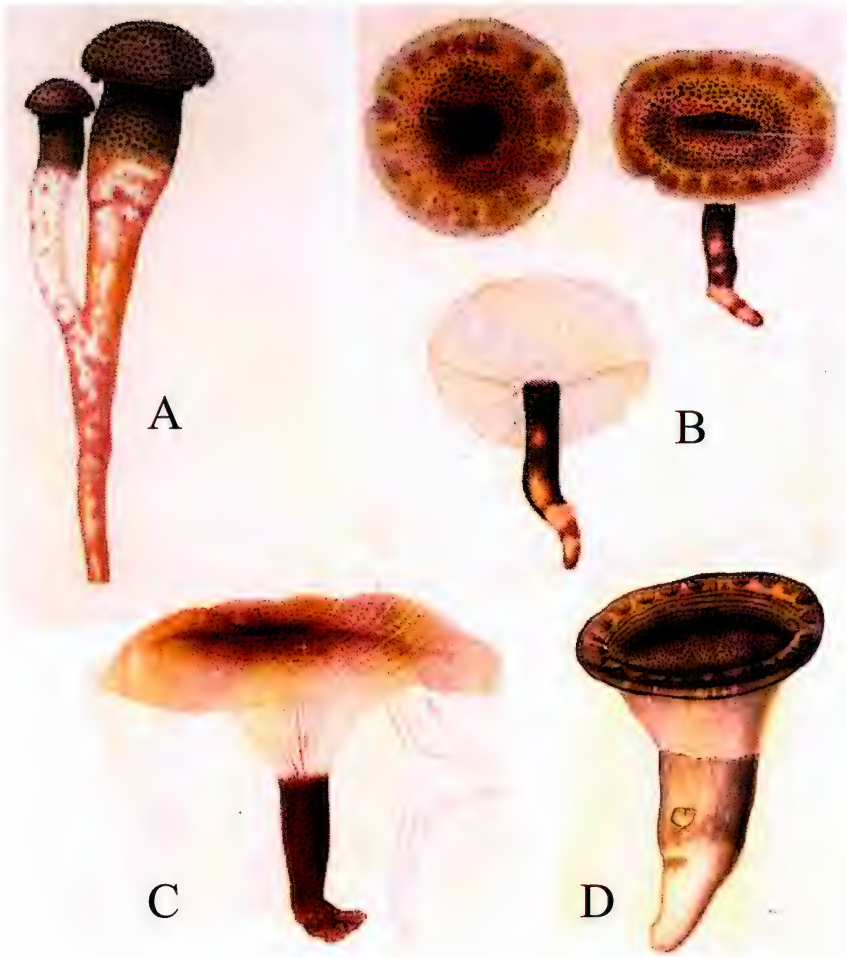

Plate 2. *Lentinus giganteus*: watercolour illustrations by Mr. William De Silva conserved at Fungal Herbarium, Horticultural Crop Research and Development Institute (HORDI), Sri Lanka [scanned by and reproduced with permission of Mrs. Srimathie Udugama, Director, Fungal Herbarium, Horticultural Crop Research and Development Institute (HORDI), Sri Lanka]. A–C: Sri Lanka, Hautane Range, on ground, July 1844, Gardner n.58 (holotype; Berkeley 1847); A: habit of young basidiocarps; B. cap surface, upper view; C: side view of mature basidiocarp. D: Sri Lanka, Peradeniya, July 1868, Thwaites n.688 (Berkeley & Broome 1873, as *L. stenophyllus*); habit of basidiocarp.

thin-walled. The large spores are not cylindrical but rather broadly ellipsoid, although the largest spores become oblong ellipsoid. Basidia (Plate 3c) 25–40 × 8–10 µm, elongate, clavate, bearing 4 sterigmata. Lamella edge sterile with a broad layer of Cheilocystidia (Plate 3b) 15–30 × 6–10 µm, more or less

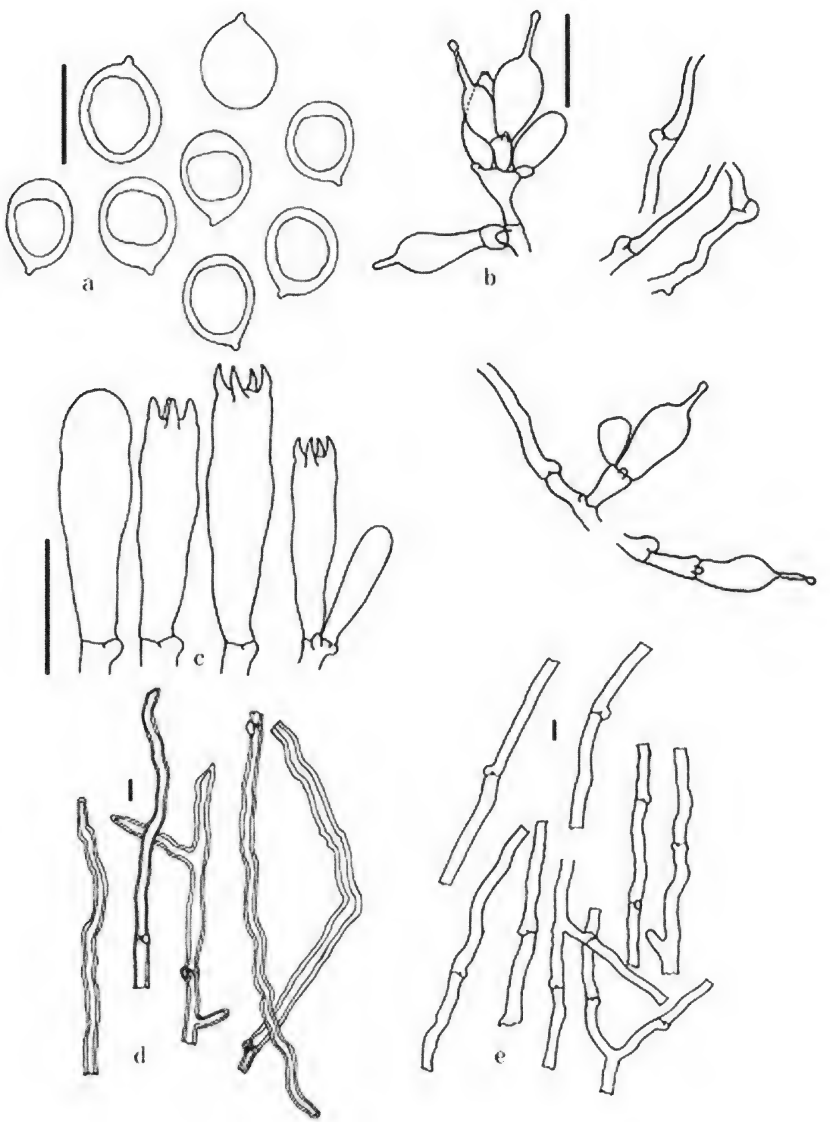

Plate 3. *Pleurotus giganteus* (mflu08 1370). a: Spores; b: Cheilocystidia; c: Basidia; d: Skeletal hyphae; e: Generative hyphae; Scale bars: a–c = 20 µm; d, e = 10 µm.

Plate 4 (to right). A. Basidiocarp of *Pleurotus giganteus* from Sri Lanka (mflu11 0018, epitype); B–E: Basidiocarps of *P. giganteus* from northern Thailand (B: mflu10 0141; C: mflu08 1371; D, E: mflu08 1370). Scale bars: A = 20 cm; B, C = 10 cm.

A
B
C
D
E

lecythiform with a ventricose base and a small capitellum (3–4 µm) subtended by a narrow neck, hyaline, thin-walled.

Ecology and distribution— solitary on buried rotten wood in rain forest. Widely distributed in Australia, China, Malay Peninsula, Sabah, Sri Lanka, Vietnam (Pegler 1983), Thailand (this study).

Additional material examined: **THAILAND**, **Chiang Mai Prov.**, Mae Taeng Distr., Ban Pha Deng, Mushroom Research Centre, 19°17.123′N 98°44.009′E, elevation 900 m, rainforest dominated by *Castanopsis armata*, *Erythrina* sp, and *Dipterocarpus* sp., 8 July 2008, Ruilin Zhao (MFLU08 1370); 21 June 2008, Samantha C. Karunarathna (MFLU10 0138); 27 June 2008, Samantha C. Karunarathna (MFLU08 1371); 6 July 2008, Samantha C. Karunarathna (MFLU08 1382); 22 July 2008, Samantha C. Karunarathna (MFLU10 0137); 10 July 2010, Olivier Raspe (MFLU10 0153); Doi Suthep-Pui National Park, Sangasabhasri Lane to Huai Kok Ma village, 18°48.62′N 98°54.60′E, elevation 1145 m, rainforest dominated by *Castanopsis* spp., *Lithocarpus polistachyus* and other trees, 9 June 2008, Samantha C. Karunarathna (MFLU10 0136); **Chiang Rai Prov.**, Highway No.110 to Mae Sai, Doi Tung, 20°17′37″N 99°48′56″E, elevation 950 m. 15 July 2009, Samantha C. Karunarathna (MFLU10 0140); 15 July 2009, Samantha C. Karunarathna (MFLU10 0141); 8 August 2009, Samantha C. Karunarathna (MFLU10 0142); 8 August 2009, Samantha C. Karunarathna (MFLU10 0143); 16 July 2010, Samantha C. Karunarathna (MFLU10 0154).

Discussion— Molecular evidence indicates that *Lentinus giganteus* is better placed in *Pleurotus*. This is supported by morphology as (1) the lamella-edge is well defined with a broad, sterile layer of differentiated cheilocystidia, similar to those found in some *Pleurotus* species; (2) basidiomes are soft in texture with short life span, similar to *Pleurotus* species; (3) the lamellae are broad and well spaced; and (4) development is metavelangiocarpic. Although Corner (1981) established *L.* subg. *Gigantopanus* for *L. giganteus*, our limited data do not support this division.

We collected 11 *Pleurotus giganteus* specimens from three sites in northern Thailand and one specimen from the original site in Sri Lanka cited in the protologue. The new Sri Lankan collection, identical to that of Corner's (1981) description and our observation of the holotype, is designated as epitype of *P. giganteus*. Molecular data groups our collections from Thailand with high bootstrap support in a single clade that clusters with sequences of *P. giganteus* from China, suggesting strain similarity. The Sri Lankan *P. giganteus* collection forms a sister group with Thai and Chinese collections with 86% bootstrap support. This suggests that the Chinese and Thai collections might have diverged from the Sri Lankan species due to geographical isolation. There are also micro-morphological differences (Table 2), although more collections are needed to confirm whether these are different taxa. Thus we maintain *P. giganteus* as a single widely variable species. Both Thai and Sri Lankan collections are more closely related to *Pleurotus* than to *Panus* and *Lentinus*. *Pleurotus australis, P. smithii, P. cystidiosus,* and *P. fuscosquamulosus* (all *Pleurotus* subg.

Table 2. *Pleurotus giganteus* morphological comparisons

Isolate/Collection	Cap (diam.) Stipe (length) (both in mm)	Spores (mm)	Basidia (mm)	Cheilocystidia (μm)
MFLU08 1370	Cap: 100–110 Stipe: 65–70	7–9 × 6–7	25–40 × 8–10	15–30 × 6–10
MFLU10 0138	Cap: 90–110 Stipe: 80–110	6.5–8.5 × 6–7	26–38 × 7.5–9.5	14–30 × 6.5–11
MFLU08 1371	Cap: 165–170 Stipe: 65–70	6.5–8.5 × 6–7	25–39 × 7.5–9.5	13.5–31 × 6–11
MFLU08 1382	Cap: 45–50 Stipe: 65–70	6.5–8.5 × 6.5–7	25.5–41 × 8–10.5	15–31 × 6–10.5
MFLU10 0137	Cap: 105–110 Stipe: 50–60	6.5–8.5 × 6–7	24–41 × 8–10	15–31 × 6.5–10.5
MFLU10 0153	Cap: 140–150 Stipe: 190–200	6.5–8.5 × 6.5–7	25–41 × 8.5–10.5	14–30.5 × 6–10
MFLU10 0140	Cap: 50–70 Stipe: 130–150	6.5–8 × 6.5–7	25–41 × 8.5–10.5	15–31 × 6.5–11
MFLU10 0143	Cap: 200–220 Stipe: 70–80	6–8 × 6–7	25–41.5 × 8–10	15–30.5 × 6–10
MFLU10 0141	Cap: 70–100 Stipe: 150–180	6.5–8 × 6.5–7.5	25–39.5 × 8.5–10	14–30.5 × 6.5–10.5
MFLU10 0142	Cap: 100–180 Stipe: 120–130	6.5–8.5 × 6.5–7	24–40 × 8.5–10	14–31 × 6.5–10.5
MFLU10 0154	Cap: 150 Stipe: 75	6.5–8.5 × 6.5–7	23.5–41 × 8–10	15.5–31 × 6.5–10
MFLU10 0136	Cap: 130 Stipe: 110	6.5–8.5 × 6.5–7	24–41 × 8–9.5	15–31 × 6.5–10.5
MFLU11 0018 (epitype)	Cap: 60–310 Stipe: 50–190	6–8 (-9) × 4.3–5.2	29–52 × 8–9.5	23–35(–38) × 6–10
K (holotype)	Cap: 50–300 Stipe: 50–180	6–8 × 4.5–5.2	30–50 × 8–9	25–35 × 6–10

Coremiopleurotus) group with all sequenced *P. giganteus* collections with 100% bootstrap support.

Pleurotus giganteus is closely related to *P.* subg. *Coremiopleurotus,* a group of edible species with high commercial value (Zervakis et al. 2004). The relationship suggests that *P. giganteus* is likely to be a good edible species, confirmed by its present consumption in Sri Lanka (Pegler 1983; Udugama & Wickramaratna 1991). *Pleurotus giganteus* is also considered edible in China (Dai et al. 2010).

The most commonly and easily cultivated mushrooms in Thailand and other southeast Asian countries are oyster mushrooms (*Pleurotus ostreatus* (Jacq.) P. Kumm.), ear mushrooms (*Auricularia polytricha* (Mont.) Sacc.), and straw mushrooms (*Volvariella volvacea* (Bull.) Singer). Other mushroom species in

Lentinula, Lentinus, Ganoderma, and *Macrocybe* (Hanko 2001, Karunarathna et al. 2011, Boa 2007) and *Agrocybe* can also be cultivated successfully but require more attention and knowledge (Boa 2007).

Wild mushrooms are one of the higher valued non-timber forest products in northern Thailand (Sysouphanthong et al. 2010, Karunarathna et al. 2011). They provide locals with seasonal food, medicine, and an alternative income while maintaining forest health (Sysouphanthong et al. 2010). The richness of wild mushrooms is also one bioindicator of ecosystem health (Dai & Yang 2009; Du et al. 2011a,b; Sysouphanthong et al. 2010; Egli 2010). Cultivated, non-mycorrhizal mushrooms (e.g., *Lentinula edodes* (Berk.) Pegler, *Pleurotus sajor-caju* (Fr.) Singer, *Flammulina velutipes* (Curtis) Singer, and species of *Agaricus, Auricularia, Pleurotus, Agrocybe,* and *Volvariella*) are available year around in northern Thai markets. However, edible wild mushrooms can be found only in the wet season from June to September (Sysouphanthong et al. 2010, Karunarathna et al. 2011). As *Pleurotus* species form a heterogeneous commercially valuable group of edibles, it is therefore desirable to try to introduce members of this genus as commercial species. Although there are many studies on cultivated and wild edible mushrooms and their nutritional value in the northern hemisphere (Aletor 1995; Latiff et al. 1996; Manzi et al. 1999, 2001; Dermirbas 2000), there is little information available concerning the taxonomy, biodiversity, and economic potential of *Pleurotus* species in the tropics. Scientific information on wild mushrooms is essential for the introduction of new species for the table (Karunarathna et al. 2011, Sysouphanthong et al. 2010).

Based on the survey of 30 locals through a questionnaire in Chiang Mai and Chiang Rai provinces, we were unable to obtain a clear idea as to whether or not they regard *P. giganteus* as an edible mushroom. *Pleurotus giganteus* is not sold in Thai markets during wet season, and most inhabitants of Chiang Mai and Chiang Rai provinces do not consume this wild edible mushroom. Even though *P. giganteus* has a very good taste (Udugama & Wickramaratna 1991), it is not yet cultivated in Thailand as a commercial mushroom.

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Diversispora clara (*Glomeromycetes*)— a new species from saline dunes in the Natural Park Cabo de Gata (Spain)

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Abstract — A new species of *Diversispora* (*Glomeromycetes*) was found in saline sand dunes of the Natural Park Cabo de Gata (Almería, Andalucía, Southern Spain) in the rhizosphere of *Asteriscus maritimus*, a plant species especially adapted to saline environments. The new fungal species forms brilliant white spores that are 79–130 × 75–125 µm and have one wall consisting of three layers. The subtending hyphae are, as typical for many *Diversispora* spp., thin-walled, hyaline, and cylindrical (or rarely constricted) and flexible and fragile below the septa separating the spore and hyphal contents. The septa form regularly at the spore bases or, less frequently, in subtending hyphae at short distances from the spore base. Phylogenetic analyses of the ITS and partial 28S ribosomal gene confirm that *D. clara* forms a monophyletic, independent clade within *Diversispora*.

Key words — *Glomus*, *Glomeromycota*, Europe, extreme environments, rDNA

Introduction

During recent studies on arbuscular mycorrhiza (AM) fungal diversity in sand dune systems of the Cabo de Gata Natural Park in Almería (Spain), a brilliant-white new glomeromycotean fungus was recovered from the rhizosphere of *Asteriscus maritimus*, a plant species characteristic of saline Mediterranean environments with elevated soil electrical conductivity. The new fungus formed spores in AM fungal bait cultures predominantly in the *Asteriscus maritimus* rhizosphere. The aims of the present study were to analyze this particular fungus applying combined morphological and molecular tools and to describe its characteristics.

Material & methods

Soil and plant sampling

Soil samples were taken in February 2010 from the rhizosphere of ten *Asteriscus maritimus* plants growing in a natural sand dune system of the Natural Park Cabo de Gata in Almería (Andalucía, Spain). The site is located at 36°44′41″N 02°07′26″E. The samples, air-dried in the laboratory, were used to analyze selected chemical soil parameters (pH, soil organic carbon, total nitrogen, soil electrical conductivity) and spore populations. The ten plants and the surrounding rhizosphere soil were also extracted and used as bait cultures for propagation of AM fungal communities indigenous to the natural sand dune system. At the site, the soil was sandy and with pH (H_2O) 8.2, organic carbon 15.3 g kg^{-1}, total nitrogen 1.9 g kg^{-1}, available P 27.0 mg kg^{-1}, and soil electrical conductivity 1.5 µS cm^{-1}.

AM fungal bait cultures

Bait cultures were established and maintained as described in Palenzuela et al. (2008, 2011) by transplanting 10 *Asteriscus maritimus* plants with their rhizosphere soils into 1 L pots and transferring them to the greenhouse at EEZ in Granada immediately after sampling. The pots were irrigated three times per week and fertilized every four weeks with Long-Aston nutrient solution (Hewitt 1966). Pure cultures of the new fungus were initiated in a mixed-culture of three *Allium porrum* L. and three *Hieracium pilosella* L. plantlets in 750 mL pots grown together at three locations in the pots. The plant rhizosphere was inoculated with 20 spores per pot and pot location. A sterile mixture of Terragreen (American aluminum oxide, Oil Dry US special, type III R; Lobbe Umwelttechnik Iserlohn, Germany) and Loess (mixture 3:1; with pH-KCl 6.2; organic carbon 0.3%; available P (Na-acetate) 2.6 mg kg^{-1}; available K (Na-acetate) 350 mg kg^{-1} was chosen as culture substrate (Oehl et al. 2002). So far, pure cultures of the new fungus have not been obtained.

Morphological analyses

AM fungal spores were separated from the soil samples by a wet sieving process (Sieverding 1991). The morphological spore characteristics and their subcellular structures were described from a specimen mounted in: polyvinyl alcohol-lactic acid-glycerol (PVLG; Koske and Tessier 1983); a mixture of PVLG and Melzer's reagent (Brundrett et al. 1994); a mixture of lactic acid to water at 1:1; Melzer's reagent; and water (Spain 1990). The spore structure terminology follows Oehl et al. (2003, 2005, 2011a) for species with glomoid or diversisporoid spore formation. Photographs (Figs 1–10) were taken with a Leica DFC 290 digital camera on a Leitz Laborlux S compound microscope using Leica Application Suite Version V 2.5.0 R1 software. Specimens mounted in PVLG and the PVLG+Melzer's mixtures were deposited at the herbaria Z+ZT (ETH Zurich, Switzerland), GDA-GDAC (University of Granada, Spain), and URM (Federal University of Pernambuco, Recife).

Molecular analyses

Five spores isolated from the trap cultures were surface-sterilized with chloramine T (2%) and streptomycin (0.02%) (Mosse 1962) and crushed with a sterile disposable micropestle in 40 µL milli-Q water (Ferrol et al. 2004). PCRs of the crude extracts

were obtained in an automated thermal cycler (Gene Amp PCR System 2400, Perkin-Elmer, Foster City, California) with a pureTaq Ready-To-Go PCR Bead (Amersham Biosciences Europe GmbH, Germany) following manufacturer's instructions with 0.4 μM concentration of each primer. A two-step PCR amplified the partial SSU, ITS1, 5.8S, ITS2 and partial LSU rDNA ribosomal fragment using the SSUmAf/LSUmAr and SSUmCf/LSUmBr primers consecutively (Krüger et al. 2009). The second PCR products were separated electrophoretically on 1.2% agarose gels stained with Gel Red™ (Biotium Inc., Hayward, CA, U.S.A.) and viewed by UV illumination. The band of the expected size was excised with a scalpel and the amplified DNA was isolated from the gel with the QIAEX II Gel Extraction kit (QIAGEN, Valencia, CA, USA), cloned into the PCR2.1 vector (Invitrogen, Carlsbard, CA, USA), and transformed into one shot TOP10 chemically competent *Escherichia coli* cells. After plasmid isolation from transformed cells, cloned DNA fragments were sequenced with vector primers (White et al. 1990) in both directions by Taq polymerase cycle sequencing on an automated DNA sequencer (Perkin-Elmer ABI Prism 373). Sequence data were compared to gene libraries (EMBL and GenBank) using BLAST (Altschul et al. 1990). The new sequences were deposited in the EMBL database under the accession numbers FR873629–FR873633.

Phylogenetic analyses: The AM rDNA ITS1+5.8S +ITS2 fungal sequences were aligned in ClustalX (Larkin et al. 2007) and edited with BioEdit (Hall 1999) to obtain a final alignment with *Acaulospora laevis* Gerd. & Trappe and *A. lacunosa* J.B. Morton as outgroups. Prior to phylogenetic analysis, the model of nucleotide substitution was estimated using Topali 2.5 (Milne et al. 2004). Bayesian (two runs over 1×10^6 generations with a burn in value of 2500) and maximum likelihood (1000 bootstrap) analyses were performed in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) and PhyML (Guindon & Gascuel 2003) respectively, launched from Topali 2.5, using the GTR + G model. Neighbor-joining (established with the model cited above) and maximum parsimony analyses were performed using PAUP*4b10 (Swofford 2003) with 1000 bootstrap replications.

Results

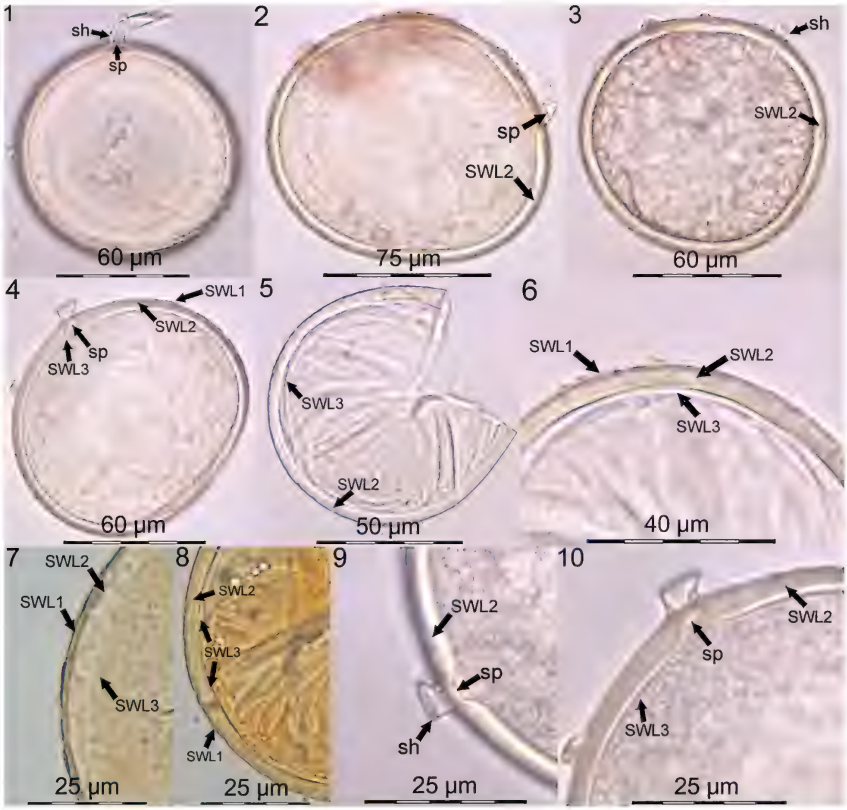
Diversispora clara Oehl, B. Estrada, G.A. Silva & Palenz., **sp. nov.** Figs 1–10

MycoBank MB 561583

Sporae albae, 79–130 × 75–125 μm, tunica tribus stratis, 4.3–8.4 μm. Stratum exterior hyalinum; stratum medium, album ad rarum ochreo-album, 2.8–5.4 μm crassum; stratum interius album. Hypha adhaerenta, 8–13 μm in diametrum. Tunica hyphae 1.0–1.8 μm crassa. Strata medium interiusque septo porum occludentes. Strata exterior mediumque flava colorantes Melzeri.

Type: Spain. Andalucía, Almería, Cabo de Gata Natural Park, sand dune, from the rhizosphere of *Asteriscus maritimus* (L.) Less. (*Asteraceae*), isolation date February 2011, by B. Estrada (**holotype**, ZT Myc 3796 [permanent slide 20-2001]; **isotypes**, ZT Myc 3797 [permanent slides 20-2003 to 20-2012], GDA-GDAC [permanent slides 20-2013 to 20-2018], URM [permanent slides 20-2019, 20-2020]).

Etymology: *clara* (Latin = clear, bright, brilliant, light), referring to the brilliant white spores.



Figs 1–10. *Diversispora clara*: 1–4. Uncrushed spores with a triple-layered spore wall (SWL1-3), a single, cylindrical subtending hypha (sh), and a septum (sp) arising directly or at some distance from the base. Figs 5–6. Spores crushed to show triple layered spore wall (SWL1-3). Figs 7–8. Spore wall structure in PVLG + Melzer's reagent. The SWL1 or SWL2 (or both) stain light to dark yellow in Melzer's, while no such staining reaction is seen on SWL3. However, the yellow stain is usually most noticeable in the spore cell contents. Fig. 9. Septum at spore base formed by SWL2 (SWL3 is not visible in uncrushed spore). Fig. 10. Septum at spore base formed by SWL3.

Spores (Figs 1–4) singly formed in rhizosphere soils, terminally on subtending hyphae, globose (80–110 µm diam.) to subglobose (79–130 × 75–125 µm), one-walled, brilliant to creamy white.

Spore wall 4.3–8.4 µm thick, three-layered (SWL1, SWL2, SWL3; Figs 5–7); outer layer (SWL1) hyaline, 0.8–1.5 µm thick, evanescent and thus often absent in mature spores; second layer (SWL2) bright to (rarely) creamy white, laminated, 2.8–5.4 µm thick (Fig. 7) in uncrushed spores (sometimes ≤ 6.6 µm in crushed spores when pressure is applied on the cover slip); inner layer (SWL3)

brilliant white, 0.7–1.5 µm thick (usually tightly adherent to SWL2 and difficult to observe when < 1.0 µm; see FIGS 5–6); both SWL1 and SWL2 generally light to dark yellow in Melzer's reagent (FIGS 7–8, with spore cell contents often a more intense yellow than the surrounding cell wall layers (FIG. 8).

SUBTENDING HYPHAE (sh) generally singly on spores; brilliant white (FIGS 1–3), cylindrical or (rarely) slightly constricted at the spore base, 4.0–8.0 µm broad tapering to 3.2–6.1 µm within 100 µm of spore base, although the distance may appear shorter (4.0–10(–25) µm) because the sh walls taper from 1.0–1.8 µm thick to 0.6–1.2 µm within the first 10 µm from the base causing the flexible fragile portion to break from the mature spore (FIGS 3, 4, 9) at a point where the septum separates the spore contents from the hyphal contents. Spore pores at the spore bases or in the sh normally closed by a septum (FIGS 1–2, 4) arising from SWL2 (FIG. 9), SWL3 (FIG. 10), or both layers (not shown); pores rarely open (FIG. 3).

DISTRIBUTION: Known only in the natural sand dune system of the Cabo de Gata Natural Park in Andalucía in the rhizosphere of *Asteriscus maritimus*.

MOLECULAR ANALYSES: Phylogenies derived from ITS (FIG. 11) and 28S (data not shown) rDNA analyses cluster the new fungus within the *Diversisporaceae* in a well-separated clade adjacent to several other *Diversispora* species, *Otospora bareae,* and *Tricispora nevadensis* (Oehl et al. 2011b).

Discussion

Our morphological analyses, in particular those of the subtending hyphae and spore bases, clearly support the new fungus in *Diversispora*, with many characters identical to those of other *Diversispora* species (e.g., *D. spurca, D. eburnea;* see Oehl et al. 2011a,b) such as the thin-walled, hyaline, cylindrical (or rarely constricted) subtending hyphae that appear flexible and fragile behind the septum that closes the pore at the spore base or in short distance from the base. In *Diversispora* species with pigmented spores, the subtending hyphae regularly change color conspicuously, becoming hyaline to white behind the septum (Oehl et al. 2011a). This color change, however, was not confirmed for *D. clara*, since the new fungus generally does not form pigmented spores.

Molecular analyses confirm the species as new: in the phylogenetic tree, *D. clara* clusters in a independent, monophyletic clade within a polyphyletic *Diversispora*. These sequence analyses also confirm unequivocally using morphology to identify diversisporoid species within the *Glomeromycota* (Oehl et al. 2011a,c).

Including *D. clara*, there are now 14 *Diversispora* spp. known in the *Glomeromycetes* (Oehl et al. 2011a,b). The 9 species that form significantly pigmented, yellow brown to brown or orange to orange brown spores are *D. arenaria* (Błaszk. et al.) Oehl et al., *D. aurantia* (Błaszk. et al.) C. Walker & A. Schüssler, *D. epigaea* (B.A. Daniels & Trappe) C. Walker & A. Schüssler,

D. insculpta (Błaszk.) Oehl et al., *D. przelewicensis* (Błaszk.) Oehl et al., *D. pustulata* (Koske et al.) Oehl et al., *D. tenera* (P.A. Tandy) Oehl et al., *D. trimurales* (Koske & Halvorson) C. Walker & A. Schüssler, and *D. versiformis* (P. Karst.) Oehl et al. (Oehl et al. 2011a,b). *Diversispora celata* C. Walker et al. forms triple-layered, ochre to ivory to pinkish cream spores (Gamper et al. 2009). Only *D. spurca* (C.M. Pfeiff. et al.) C. Walker & A. Schüssler, *D. eburnea* (L.J. Kenn. et al.) C. Walker & A. Schüssler, and *D. gibbosa* (Błaszk.) Błaszk. & Kovács form hyaline to subhyaline spores that are, however, never brilliant-white as observed for *D. clara*. Moreover, *D. spurca* and *D. eburnea* have bi-layered spore walls (Kennedy et al. 1999), *D. gibbosa* has a five-layered wall (Błaszkowski 1997), and *D. clara* has a three-layered wall.

In the past, large-spored or sporocarpic AM fungi were described from sand dune systems, such as *Gigaspora*, *Scutellospora*, *Pacispora* or *Glomus* species that were generally easy to isolate and recognize from field samples (Koske & Gemma 1995, Gemma et al. 1989, Błaszkowski 1994). Likewise in the current Cabo de Gata Natural Park sand dune system, where *Funneliformis coronatus* (Giovann.) C. Walker & A. Schüssler, *F. mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler, *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders, *Racocetra persica* (Koske & C. Walker) Oehl et al., and *Glomus macrocarpum* Tul & C. Tul. were identified (Estrada, unpublished). When sand dune AM fungal communities were maintained and reproduced in bait cultures, small-spored *Glomus* spp. were also sometimes detected (e.g. Błaszkowski et al. 2009a,b, 2010). It was supposed that species with small, quickly degrading spores were difficult to recover or identify only from field samples. This might also be true for *D. clara*, even though its laminated wall structure is clearly persistent. It will be interesting to see whether future taxonomists will be able to identify the new species directly from field samples, now that the existence and morphology of this unique, brilliant-white, conspicuous but small-spored species is known. Morphological spore and molecular root and spore analyses will hopefully tell us more about the ecology and biogeography of this fungus that is thus far known only from a single *Asteriscus maritimus* rhizosphere in the Natural Park Cabo de Gata of Almería in southern Spain.

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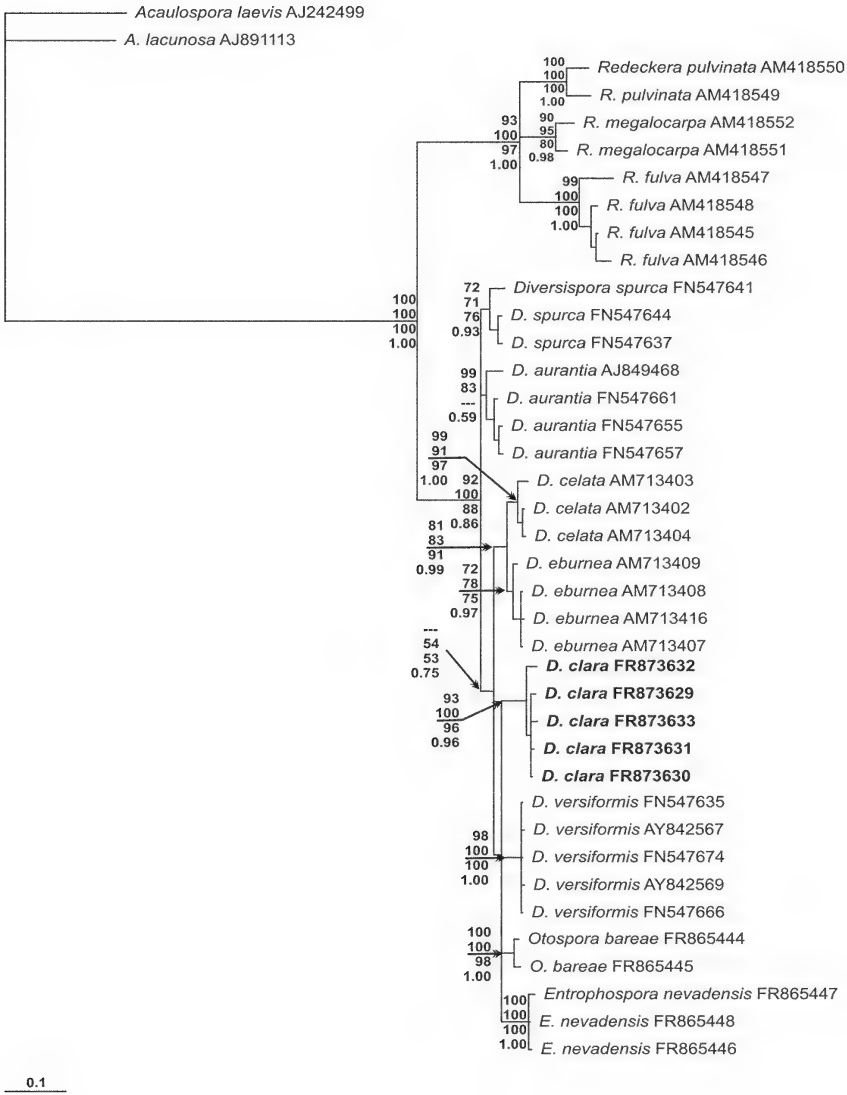

FIG. 11. *Diversisporaceae.* ITS rDNA-based phylogenetic tree rooted by *Acaulospora laevis* and *A. lacunosa*. Sequences are labeled with database accession numbers. Support values are from neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and bayesian analyses. *Diversispora clara* sequences are in bold. Only topologies with ≥ 50% bootstrap values are shown. (Consistency Index = 0.72; Retention Index = 0.84).

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Asterophora salvaterrensis (*Basidiomycota, Agaricales*), a new species from Galicia (Spain)

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Abstract—*Asterophora salvaterrensis*, collected in *Pinus pinaster* forests in Galicia (northwest Iberian Peninsula), is described as new and compared with other known *Asterophora* species. The new species is distinguished by its zonate greenish brown to brownish ochre pileus with whitish margin that blackens, decurrent obtuse furfuraceous grey lamellae, furfuraceous brown to blackish stipe, black context, nondistinctive odor and taste, and small basidiospores. A key to all known *Asterophora* species is provided.

Key words—*Lyophyllaceae,* parasitic fungi, taxonomy

Introduction

Mycological studies conducted during the last decades in the forests of maritime pine (*Pinus pinaster* Aiton) in the Miño river basin of Galicia (northwestern Spain) have produced interesting discoveries, such as the new species, *Sparassis miniensis* Blanco-Dios & Zheng Wang (Blanco-Dios et al. 2006). In this territory, several samples of a remarkable species of *Asterophora* Ditmar were recently collected on a russulaceous host (*Russula* sp.).

Species of *Asterophora* (*Lyophyllaceae)* are among the few agarics that grow on basidiomata of other fungi (the most common hosts are russulaceous species, mainly in *Lactarius* Pers. and *Russula* Pers.). The genus is further distinguished by the production of chlamydospores. *Asterophora* is phylogenetically placed in *Lyophyllaceae* (Matheny et al. 2006).

Today, only three well documented species of *Asterophora* are accepted: *A. lycoperdoides* (Bull.) Ditmar, *A. parasitica* (Pers.) Singer, and *A. mirabilis* (T.W. May) Redhead & Seifert (May & Fuhrer 1995; Redhead & Seifert 2001a,b; Vizzini 2009). Our collections, which do not fit the morphological concept of any of these taxa, are described here as a new species.

A key to the known species of *Asterophora* is provided.

Materials & methods

Macro- and micromorphological descriptions are based on observations of both fresh and dried specimens. L = number of lamellae; Q = quotient of length /width of an individual spore and avQ is the average Q. All the micromorphological structures were measured in Melzer's or 3% KOH, except for the pileipellis and stipitipellis hyphae, which were measured in 10% ammonia. Spore measurements do not include the hilar appendage. The length of intercalary chlamydospores was measured from the septum of one subtending hypha to the septum of the other; the length of terminal chlamydospores was measured from basal septum to apex. All specimens, including the holotype, are kept in LOU-Fungi herbarium (Centro de Investigación Forestal de Lourizán, Pontevedra, Spain).

Taxonomy

Asterophora salvaterrensis Blanco-Dios, **sp. nov.** PLATE 1, FIG. 1

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Pileus 0.5–4 mm latus, planus leviter depressus, non hygrophanus, haud traslucenter striatus, zonatus, ab brunneo-viridulus ad brunneo-ochraceus, ad medium brunneus, aliunde albidus, nigrescens. Lamellae decurrentes, dispersae, obtusatae, furfuraceae, griseae. Stipes 1.5–10 mm longus, 0.5–1.5 mm latus, plenus, furfuraceus, brunneus vel brunneo-nigrescens. Caro nigrescens, parce conspicua. Odor et sapor haud notabiles. Sporae 2.5–3.2(–4.5) × (1.2–)1.8–2.5 µm, Q = 1.29–2, ellipsoidaeae vel oblongae, laeves. Basidia tetrasporigera, fibulata. Acies lamellarum fertilis. Chlamydosporae (12–)13–17.5(–20.5) × (7.3–)8.5–10.5(–11.5) µm Q=1.26–2, ellipsoidaeae vel oblongae, laeves. Fibulae praesentes. Ad basidiomata corruptas Russulae nigricantis.

TYPE: Spain: Pontevedra, Salvaterra de Miño, Leirado. Collected by J.B.Blanco-Dios, 1-XII-2009 (**Holotype:** LOU-Fungi 19491).

ETYMOLOGY: *salvaterrensis*, from the municipality of Salvaterra de Miño (Galicia, Spain).

PILEUS 0.5–4 mm diam., plano-convex to flattened with depressed centre, margin first involute, then broadly wavy, not hygrophanous or translucently striate, zonate, brown at the disc, greenish brown to brownish ochre elsewhere with the margin whitish, blackening with age or handling. LAMELLAE ≤ 1 mm broad, decurrent, scattered (L = 8–10), thick, not forked, entirely furfuraceous over a grey pale or grey colour, edge concolourous, even, obtuse, lamellulae absent. STIPE 1.5–10 × 0.5–1.5 mm, central, even or a bit expanded above or attenuate, filled, entirely furfuraceous over a brown or blackish brown surface. CONTEXT very thin, black when exposed, finally brown to ochre. ODOR AND TASTE nondistinctive. SPORE PRINT whitish.

BASIDIOSPORES 2.5–3.2(–4.5) × (1.2–)1.8–2.5 µm, Q = 1.29–2, avQ = 1.47, (n = 30), sparse, ellipsoid to oblong, smooth, thin-walled, not amyloid. BASIDIA (10–)13–19 × 4–7 µm, sparse, broadly clavate, 4-spored, sterigmata up to 2.5 µm long, siderophilous. CHLAMYDOSPORES (12–)13–17.5(–20.5)× (7.3–)8.5–10.5(–11.5) µm, Q = 1.26–2, avQ = 1.56, numerous in the lamellar trama,

PLATE. 1. *Asterophora salvaterrensis* (holotype). Habit.
Photos by Amancio Castro.

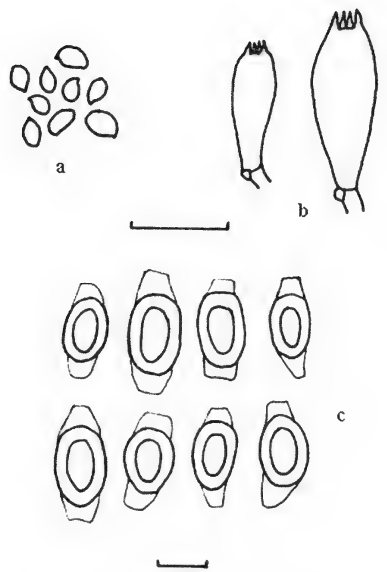

Fig. 1. *Asterophora salvaterrensis* (holotype).
a. basidiospores; b.basidia; c. chlamydospores. Scale bars = 10 µm.

in the pileal trama above the lamellae, and on the pileus surface, numerous also in the host tissue, intercalary or terminal, smooth, ellipsoid to oblong, hyaline then pale yellow, at length with an inner wall up to 0.5 µm thick which excludes a segment adjacent to each subtending hypha (the basal portion of terminal chlamydospores is similarly excluded), enclosing contents which are granular and with small and large, refractive droplets. Cystidia not seen. Pileipellis a cutis, consisting of parallel, cylindrical hyphae, 1.5–10 µm diam., thin walled, radially arranged, with ochre intracellular pigment. Stipitipellis a cutis, composed of parallel, cylindrical hyphae, 1.5–9 µm diam., thin walled. Clamp connections present in all tissues.

Ecology & distribution— On blackened and rotten *Russula nigricans* basidiomata, growing together with *Asterophora lycoperdoides* and *A. parasitica* in a *Pinus pinaster* forest. Known only from a single locality in Spain. November–December.

Collections examined: **SPAIN**: **Pontevedra**: Salvaterra de Miño, Leirado, 29TNG4565, 110 m, forest of *Pinus pinaster*, on basidiomata of *Russula nigricans*, 1.XII.2009, leg. J.B.Blanco-Dios, LOU-Fungi 19491 (holotypus); 24.XI.2009, leg. J.B.Blanco-Dios, LOU-Fungi 19490.

Other collections examined: ***Asterophora lycoperdoides*** – **SPAIN**: **A Coruña**: Brión, Adoufe, 29TNH2645, 70 m., on basidiomata of *Russula nigricans*, 19.XII.1977, leg. L. Cabo Rey, LOU-Fungi 0114; Santiago, bosque de la Condesa, 29TNH3747,

250 m., on basidiomata of *Russula nigricans*, 23.X.1975, leg. L. Freire, LOU-Fungi 0112. **Pontevedra**: A Estrada, Arnois, 29TNH4834, 60 m., on basidiomata of *Russula nigricans*, 23.X.1975, leg. L. Freire, LOU-Fungi 0116; Vilagarcía de Arousa, 29TNH1916, 20 m., on basidiomata of *Russula* sp, 18.XI.1987, leg.: E.Valdés-Bermejo, LOU-Fungi 0113.

Asterophora parasitica – **SPAIN: A Coruña**: Brión, Adoufe, 29TNH2645, 70 m., on basidiomata of *Russula nigricans*, 12.XI.1981, leg. L. Cabo Rey, LOU-Fungi 0117; Cambre, Cecebre, Fraga de Cecebre, 29TNH5794, 100 m., on basidiomata of *Russula* sp., 17.XI.1990, leg. L. Freire, LOU-Fungi 4557; Puebla do Caramiñal, Cabío, 29TNH0514, 10 m., on basidiomata of *Russula densifolia* Secr. ex Gillet, 25.XI.1989, leg. M. Martínez Campos, LOU-Fungi 6930; on basidiomata of *Russula nigricans*, 08.XII.1984, leg. L. Freire & M. L. Castro, LOU-Fungi 0121. **Lugo**: Mondoñedo, Couboeira, 29TPJ3215, 100 m., on basidiomata of *Russula* sp., 3.X.1992, leg. S. Cabanela, LOU-Fungi 5844.

Comments. *Asterophora salvaterrensis* is unique with respect to the other known *Asterophora* species —*A. lycoperdoides, A. parasitica, A. mirabilis*— by the following combination of features: (i) the zonate pileus which is brown at the disc, greenish brown to brownish ochre towards margin and whitish at the margin, (ii) the pileus-surface turning blackish with age or handling, (iii) the decurrent lamellae, which are furfuraceous and grey-tinged, (iv) the furfuraceous stipe-surface over a brown or blackish-brown cortical layer, (v) the context blackening when exposed and lacking a distinct smell and taste. From the micromorphological point of view the small, ellipsoid to oblong basidiospores associated with large, smooth, ellipsoid to oblong chlamydospores are typical and diagnostic. Some stellate chlamydospores (from basidiomata of *Asterophora lycoperdoides*),13–16.5 × 12–15 µm, were seen in the pileipellis, lamellae, and stipitipellis surface.

Asterophora lycoperdoides differs by having stellate chlamydospores, formed in the upper pileal trama. Additionally, this species frequently does not produce basidiospores, has a globose to pulvinate pileus with a powdery surface, and shows often only rudimentary lamellae. *Asterophora parasitica* also frequently does not produce basidiospores, has a convex to umbonate pileus with a typically silky surface and obtuse but comparatively well-formed lamellae. Both of these species cover a similar geographic range spanning through North America, Europe, North Africa, East Asia, and Papua New Guinea (Corner 1966, Horak 1980, Singer 1986). *Asterophora mirabilis,* known from Australia (Victoria and Tasmania), is similar to *A. parasitica*, but differs in producing stellate chlamydospores that are numerous in the lamellar trama and pileal trama above the lamellae and sparse to absent in the upper pileal trama and on the pileus surface (May & Fuhrer 1995).

From the taxonomic point of view, owing to the fact that it produces only smooth chlamydospores, *Asterophora salvaterrensis* seems to be close to *Asterophora parasitica* from which it is easily distinguished by greenish-brown to greenish-ochre zonate pileus and much smaller basidiospores.

Key to the known species of *Asterophora*

1a. Chlamydospores stellate 2
1b. Chlamydospores smooth 3
2a. Pileus surface powdery; chlamydospores formed in the upper pileal trama ..... ***A. lycoperdoides***
2b. Pileus smooth; chlamydospores formed in the lamellar trama and pileal trama above the lamellae..... ***A. mirabilis***
3a. Pileus white or grey with a silky surface, not zonate; basidiospores 5–6 × 3–4 µm ..... ***A. parasitica***
3b. Pileus brown-greenish-ochre, zonate; basidiospores 2.5–3.2 × 1.8–2.5 µm..... ***A. salvaterrensis***

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Amicodisca castaneae sp. nov. (*Hyaloscyphaceae, Helotiales*) on Japanese chestnut bur

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Abstract— *Amicodisca castaneae* sp. nov. was collected on Japanese chestnut bur. It differs from all known members of *Amicodisca* by having brownish apothecia and smaller asci and ascospores. A key to the accepted *Amicodisca* species is given.

Key words— *Castanea crenata*, discomycetes, species nova, taxonomy

Introduction

Svrček (1987) established a monotypic genus *Amicodisca* Svrček, typified by *Amicodisca brdensis* (Velen.) Svrček (≡ *Dasyscyphus brdensis* Velen.), which is characterized by yellowish to olivaceous colored excipulum and hairs, ascospores bearing lemon-yellow pigment dissolving in NH_4OH or KOH solutions, and fimbriate dehiscence of the ascus pore. *Amicodisca viridicoma* (Peck) J.H. Haines, *A. svrcekii* Raitv. & Huhtinen, and *A. groenlandica* Raitv. were later additionally described (Haines 1989; Raitviir 2001, 2003). Since Huhtinen (1994) proposed *A. virella* (P. Karst.) Huhtinen (with its earlier basionym) as the correct name for the type species, and Raitviir (2004) listed *A. viridicoma* as a synonym of *A. virella,* there are three species now recognized.

In the course of extensive survey on the fungal biodiversity in Korea, an interesting discomycete with small apothecia surrounded with yellowish hairs was collected on fallen burs of Japanese chestnut (*Castanea crenata* Siebold & Zucc.). Its gross morphology and microscopic features fit well with those of *Amicodisca,* but it is clearly different from the known species in having remarkably smaller asci and ascospores and by host preference. Here, we describe and illustrate this fungus as new to science.

Materials & methods

Fresh materials were primarily mounted in distilled water to confirm the natural colors of their microstructures. Dried materials were revived in 3–10% aqueous KOH. Amyloid reactions were tested by Melzer's reagent (MLZ) or Lugol's solution (IKI) without KOH pretreatment. Olympus BX50 microscope equipped with a drawing tube (Olympus U-DA) was used for measurements and line drawings. Structures were measured at 1000×; measurements are reported as follows: minimum–maximum (length) × minimum–maximum (width) [mean length ± standard deviation × mean width ± standard deviation, Q (l/w ratio) = average ± standard deviation]. All specimens examined are deposited in the Herbarium of Korea University, Seoul, Korea (KUS) or National Museum of Nature and Science, Tsukuba, Japan (TNS).

Taxonomy

Amicodisca castaneae J.G. Han, Hosoya & H.D. Shin, **sp. nov.** FIGS. 1–2

MycoBank 561134

Apothecia sessilia, bruneta, receptaculo dense sulphureo longipiloso. Pili cylindro-conique, 2–5-septati, tenuiter tunicatis, 63–107 × 2–3 µm. Excipulum ectale ex textura angularis ad prismatica compositur. Asci non uncinati, cinereae, 38–57 × 4–5.2 µm. Sporae ellipsoidea ad clavato-ellipsoideae, hyalinae ad cinereae, 4.8–7 × 1.4–2 µm. Ab Amicodiscae svrcekii *ascosporis brevius et pili septatae differens.*

TYPE: The inner surface of chestnut bur (*Castanea crenata*), Korea, Goesan, Songnisan National Park, 36°44′42.54″N, 128°53′53.33″E, 320 m a.s.l., 5 Oct. 2007, J.G. Han & H.D. Shin (KUS-F51917 **Holotype**; TNS-F32105 **Isotype**).

ETYMOLOGY: The specific epithet refers to the generic name of the host.

APOTHECIA superficial, scattered to gregarious, developed on well-developed anchoring hyphae, broadly sessile; RECEPTACLE at first globose to cupulate, then becoming discoid, externally covered by yellowish white hairs, turning reddish-brown when dry; DISC up to 3 mm in diameter, greyish-brown to chestnut when fresh, turning dark brown to dark grey when dry; HAIRS cylindric-conical, straight, gradually tapering toward the apex, hyaline to yellowish, 2–5-septate, thin-walled, smooth, apex not sharply pointed, up to 3 µm wide near the base, 63–107 µm long; ECTAL EXCIPULUM yellow to light brown, composed of thin-walled angular to rectangular cells, 7–12 × 4–7 µm; ASCI arising from simple septa, cylindric-clavate, yellowish, 8-spored, apical pore blued in MLZ and IKI without KOH pretreatment, 38–57 × 4–5.2 µm (45.2 ± 4.19 × 4.5 ± 0.41 µm, n = 50); ASCOSPORES biseriate, ellipsoid to clavate or ovoid, hyaline to light yellow, aseptate, eguttulate, occasionally containing a central guttule, smooth, 4.8–7 × 1.4–2 µm (5.7 ± 0.69 × 1.6 ± 0.18 µm, Q = 3.6 ± 0.62, n = 120); PARAPHYSES filiform, hyaline to yellowish, septate, slightly bent, sometimes branched, slightly exceeding the asci, up to 1.5 µm wide.

ADDITIONAL SPECIMENS EXAMINED – the inner surface and spines of chestnut bur: KOREA, WONJU, Chiaksan National Park, Geumdae valley, 37°17′44.44″N, 128°01′10.34″E, 370 m a.s.l., 9 Sep. 2006, J.G. Han & H.D. Shin (KUS-F51377);

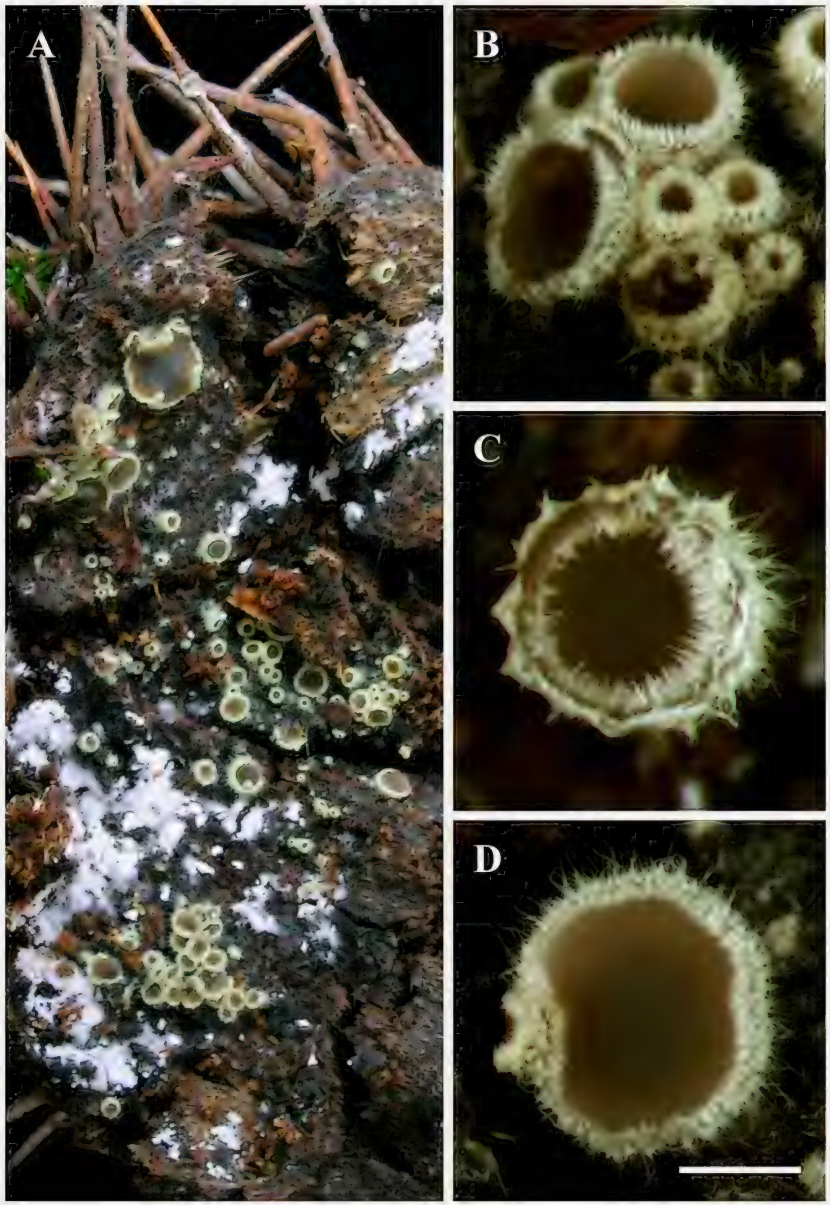

Fig. 1. *Amicodisca castaneae* (holotype KUS-F51917). A: Fruiting bodies on a fallen bur of *Castanea crenata*, B: Sessile apothecia densely gregarious, C: An immature apothecium, globose to cupulate, D: A mature apothecium, expanded. Scale bar: C–D = 1 mm.

Boryeong, Oseosan recreation forest, 36°26′31.52″N 126°40′3.2″E, 240 m a.s.l., 27 Oct. 2007, J.G. Han & H.D. Shin (KUS-F51993).

Discussion

Amicodisca is a small group of hyaloscyphaceous discomycetes characterized by a lemon-yellow colored excipulum, hairs, and ascospores (Svrček 1987). Although its dark globose ectal cells and translucent greyish hymenium suggest a relationship to *Dermateaceae*, many authors (Svrček 1987; Haines 1989; Huhtinen 1994; Huhtinen & Læssøe 2001; Raitviir 2003, 2004) have preferred to assign the genus to the *Hyaloscyphaceae* based on the presence of distinct hairs. *Dematioscypha* Svrček is similar to *Amicodisca* in having a dark excipulum and cylindric hairs with tapering apices, but differentiate the genus by its hyaline hairs with glassy apices, inamyloid ascal pore, and association with a *Haplographium* anamorph. Superficially, it is also reminiscent of *Dennisiodiscus* Svrček in sessile apothecia with greyish hymenium and bright colored surrounding hairs, but differs in hairs encrusted by reddish brown granules and growing on culms or leaves of monocotyledonous hosts.

Macroscopically, the present fungus is easily discriminated from other species by relatively large apothecia (≤ 3 mm diam.) with brownish hymenium. In contrast, all known species of *Amicodisca* produce greyish or olivaceous apothecia and are usually smaller than 1 mm in diameter. *Amicodisca castaneae* is microscopically closest to *A. svrcekii* and *A. virella*, but their larger ascospores (8–11 and 17–25 µm long, respectively) and slightly narrower paraphyses (0.8 and 1 µm broad, respectively) are different. *Amicodisca groenlandica* strikingly differs in much larger asci (92–116 × 12–15 µm) and ascospores (20–27 × 3–5 µm).

The specificity of *A. castaneae* to chestnut burs seems to be another notable feature. Hitherto all known *Amicodisca* species have been collected on woody substrates (Raitviir 2004), such as *Alnus, Betula, Salix, Sambucus,* and other deciduous trees.

A key to the accepted species of the genus is provided below.

Key to the accepted species of *Amicodisca*

1a. Fruiting bodies formed on chestnut bur; disc brownish ..... ***A. castaneae***
1b. Fruiting bodies formed on woods; disc greyish or olivaceous **2**
2a (1b). Disc pale greenish-olivaceous when fresh, becoming dark green when dry; asci longer than 80 µm ..... ***A. groenlandica***
2b. Disc greyish when fresh and dry; asci shorter than 80 µm **3**
3a (2b). Ascospores 8–11 × 1.8–2.5 µm ..... ***A. svrcekii***
3b. Ascospores 17–25 × 4–6 µm ..... ***A. virella***

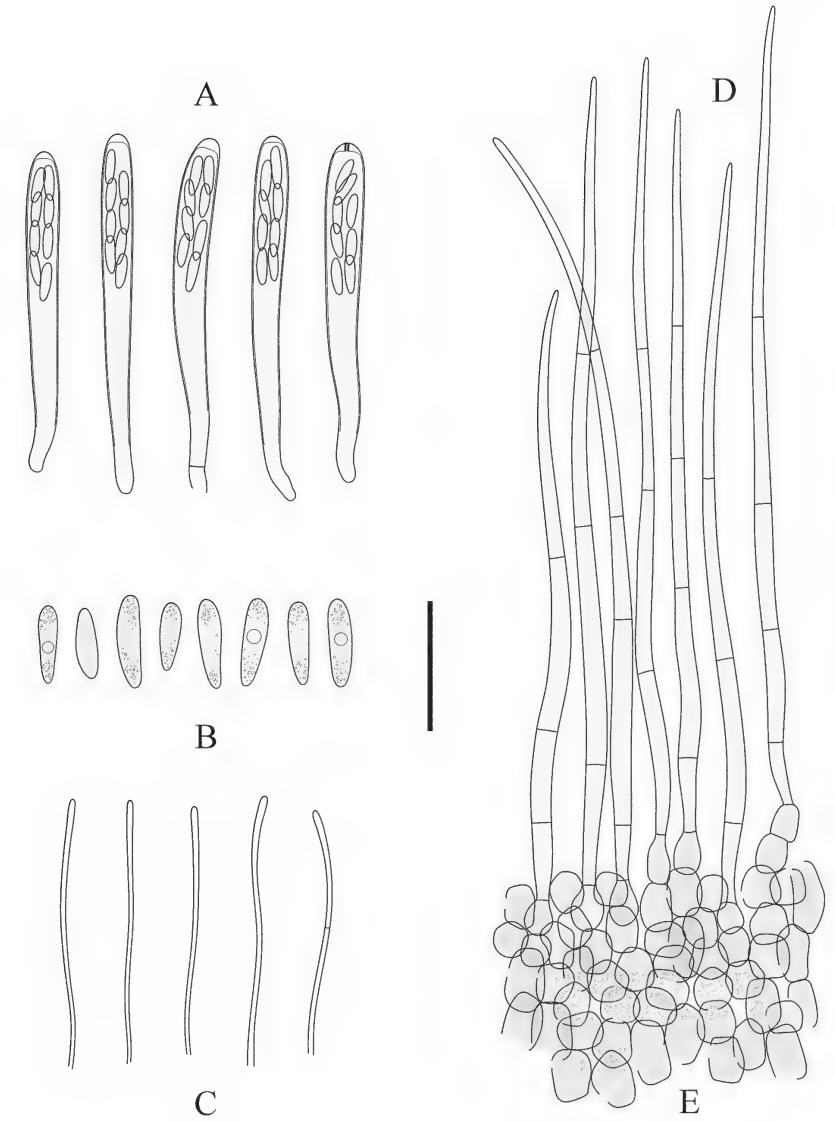

Fig. 2. *Amicodisca castaneae* (holotype KUS-F51917). A: 8-spored asci, note on the apical pore blued in MLZ, B: Ellipsoid to ovoid ascospores with pale yellowish pigments, C: Filiform paraphyses, D: Cylindric hairs with tapering apices, E: Brownish ectal excipulum composed of texture angularis to prismatica. Scale bars: A and C–E = 20 μm, B = 10 μm.

Acknowledgements

The authors are grateful to Dr. Seppo Huhtinen (Herbarium, University of Turku, Finland) and Dr. Peter Johnston (Landcare Research, New Zealand) for kindly reading the manuscript and suggesting appropriate revisions. This work was supported by a grant from Regional Subgenebank Support Program of Rural Development Administration, Republic of Korea.

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Pseudocercospora epidendri sp. nov. on the neotropical orchid, *Epidendrum secundum* from Brazil

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Abstract — A new leaf spot disease was observed on the orchid species *Epidendrum secundum* in "high altitude grasslands" of Araponga, Minas Gerais State, Brazil. Inoculation tests on healthy plants confirmed the pathogenicity of this fungus. *Pseudocercospora epidendri* sp. nov., the causal agent of the leaf spot disease of *E. secundum*, is described, illustrated, and compared with allied *Pseudocercospora* species on hosts of the family *Orchidaceae*.

Key words — cercosporoid hyphomycetes, phytopathology, plant disease, tropical fungi

Introduction

Epidendrum secundum is an orchid species with epiphytical or terrestrial habitat, characteristic of the Brazilian "high altitude grasslands" (Plate 1) and the Brazilian Cerrado (Caiafa & Silva 2005, Neto et al. 2007). In 2002, exploratory research was begun with the goal of describing the phytopathogenic mycodiversity associated with *Orchidaceae* in the state of Minas Gerais (Pereira et al. 2002, Pereira & Barreto 2004, Silva & Pereira 2007, 2008, Silva et al. 2008, Lopes et al. 2009, Pereira & Silva 2009). During an initial botanical survey in the "high altitude grasslands" of Parque Estadual da Serra do Brigadeiro in Araponga in Minas Gerais State, Brazil, samples of the orchid *E. secundum* with leaf spots caused by a cercosporoid hyphomycete were collected (Plate 2–3). Our morphological characterization showed that the leaf-spotting fungus represented a new species of *Pseudocercospora,* which we describe, illustrate, and discuss below.

Material & methods

Samples of infected *E. secundum* leaves were collected, photographed (SONY DSC-H9 digital camera), dried in a plant press, and deposited at the herbarium of Universidade Federal de Viçosa (VIC). Fungal samples were removed from fresh

Plate 1. Terrestrial *Epidendrum secundum* in the "high altitude grasslands" of the Parque Estadual da Serra do Brigadeiro, Araponga, State of Minas Gerais, Brazil.

leaf spots under an Olympus SZX7 stereomicroscope and mounted on glass slides with lactophenol. Material was also hand sectioned for stromata observation and measurements. Structures were observed, measured (30 of each structural type), and illustrated under 400× magnification using an Olympus BX 50 light microscope fitted with a drawing tube. To determine pathogenicity, the fungus was isolated directly onto PDA, brought into pure culture, and grown at 27°C for 20 days. Culture disks taken

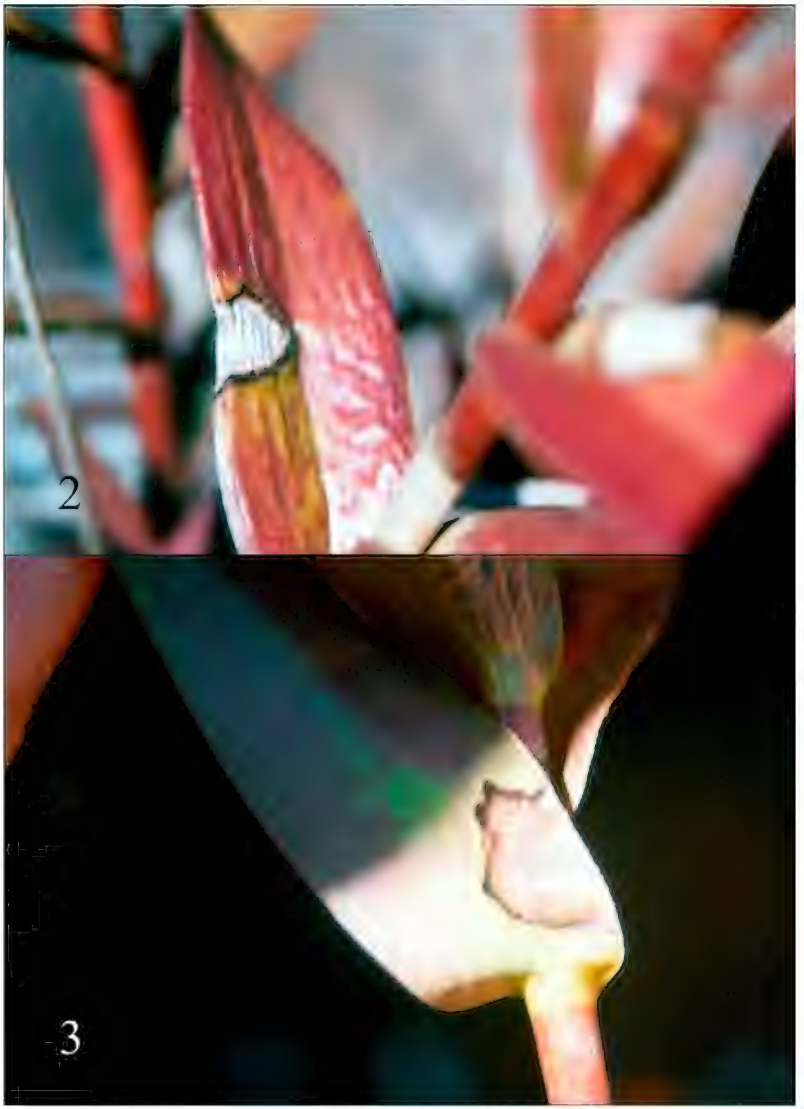

Plate 2-3. *Pseudocercospora epidendri*. Detail of infected leaf, showing delimited and irregular necrotic spots. 2. Adaxial leaf surface. 3. Abaxial leaf surface.

from colony borders were used to inoculate four healthy young and mature leaves of three *E. secundum* plants. The inoculated plants were maintained in moist chambers for three days and then transferred to a greenhouse at 25°C and 70–80% humidity. Healthy leaves, on which only PDA plugs were placed, served as control. This assay was carried in three replications.

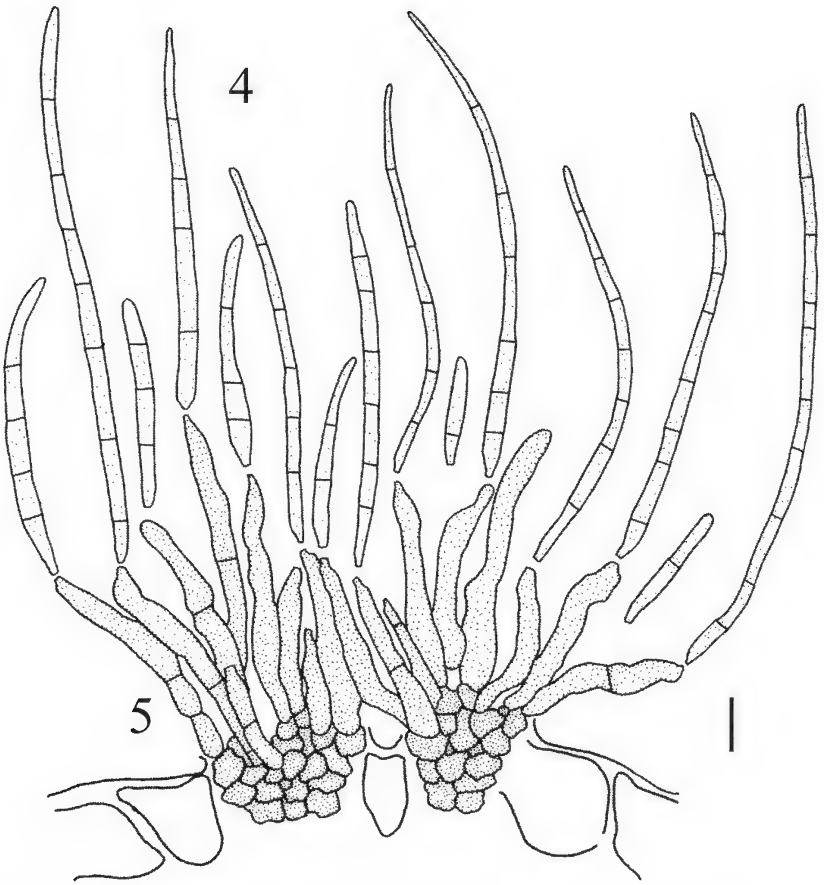

Plate 4-5. *Pseudocercospora epidendri* (VIC 30553, holotype). 4. Pigmented conidiophores with inconspicuous conidiogenous cells. 5. Solitary, pluriseptate conidia, with inconspicuous unthickened and not darkened scars. Bar: 10 μm.

Taxonomy

Pseudocercospora epidendri Meir. Silva & O.L. Pereira, **sp. nov.** Plate 4–5

MycoBank 519630

Differt a Pseudocercospora odontoglossi *conidiphoris non ramosae et brevioribus, 10–57 × 2–6 μm; conidiis brevioribus 11–86 × 1–3 μm.*

Holotype: BRAZIL, Minas Gerais, Araponga, Parque Estadual da Serra do Brigadeiro, on leaves of *Epidendrum secundum* Jacq. (*Orchidaceae*), 08 Jan. 2008, O. L. Pereira (VIC 30553, **holotype**). Ex-type culture OLP 30553 (Universidade Federal de Viçosa).

Etymology: referring to the host genus *Epidendrum*.

Plate 6. Necrotic symptoms on leaves of *Epidendrum secundum* 8 days after inoculation with *Pseudocercospora epidendri*.

Leaf spots distinct, scattered over leaves, amphigenous, irregular, pale brown, delimited by a black margin on abaxial and adaxial leaf surfaces, 3–12 mm diam. Stromata well-developed, immersed, becoming erumpent, brown, 47.5–166 µm wide and 57–213 µm high. Caespituli commonly hypophyllous, brown. Conidiophores fasciculate, brown, becoming paler towards apex, unbranched, smooth, 0-2 septate, subcylindrical, straight to curved, arising from cells of a well-developed stroma, 10–57 × 2–6 µm. Conidiogenous cells terminal, integrated, pale brown, smooth 3–4.5 × 2–3.5 µm. Conidiogenous loci inconspicuous, not darkened, unthickened. Conidia solitary, acicular to obclavate, 11–86 × 1–3 µm, 1–9 septate, straight to curved, pale brown, smooth, guttulate, base rounded to long obconically truncate hila unthickened, not darkened.

Cultures on PDA slow growing, 1.5–2.0 cm after 30 days at 27°C, stromatic and immersed in center, grayish aerial mycelium, black reverse, no sporulation.

Comments — Dark sunken necrotic symptoms were detected 8 days after artificial inoculation on all inoculated leaves, with structures formed 20–30 days after inoculation. Uninoculated control leaves, on which only PDA plugs were placed, remained healthy. The same fungus was then re-isolated, from the symptom in the inoculation assay (Plate 6). Thirteen cercosporoid species are known to occur on orchidaceous hosts: *Cercospora angraeci* Feuilleaub.

Table 1. *Pseudocercospora* spp. known to occur on *Orchidaceae.*

Species	Conidiophores (µm)	Conidia (µm)	Caespituli	Host genera
P. cypripedii	10–40 × 3–5	30–150 × 3–5	mainly epiphyllous	*Cypripedium*
P. dendrobii	10–100 × 2–5	15–80 × 2–4.5	hypophyllous	*Dendrobium*
P. peristeriae	10–60 × 2.5–6	40–100 × 2–5	mainly hypophyllous	*Peristeria*
P. odontoglossi	50–200 × 3–5.5	35–100 × 3–5	hypophyllous	*Cattleya*, *Cymbidium*, *Dendrobium*, *Epidendrum*, *Laelia*, ×*Laeliocattleya*, *Odontoglossum*
P. epidendri	10–57 × 2–6	11–86 × 1–3	mainly hypophyllous	*Epidendrum*

& Roum., *C. cephalantherae* Ondřej & Zavřel, *C. epidendronis* Bolick, *C. epipactidis* C. Massal., *C. eulophiae* M.S. Patil, *C. habenariicola* Meeboon et al., *Ramularia epipactidis* U. Braun & Rogerson, *Pseudocercospora cypripedii* (Ellis & Dearn.) U. Braun & Crous, *P. dendrobii* Goh & W.H. Hsieh [= *P. dendrobii* (H.C. Burnett) U. Braun & Crous, nom. illegit.], *P. odontoglossi* (Prill. & Delacr.) U. Braun, *P. peristeriae* (H.C. Burnett) U. Braun & Crous, *Stenella orchidacearum* U. Braun & Sivap., and *S. cyrtopodii* Dorn.-Silva et al. (Chupp 1954, Hsieh & Goh 1990, Crous & Braun 2003, Braun & Crous 2007, Dornelo-Silva et al. 2007, Meeboon et al. 2007). *Pseudocercospora epidendri* is the fifth *Pseudocercospora* species known to occur on the *Orchidaceae* (Table 1) and the second known to occur on *Epidendrum* (*P. odontoglossi* was the first reported to occur on that genus.) *Pseudocercospora cypripedii* and *P. odontoglossi* can be distinguished from *P. epidendri* by their longer and wider conidia (Chupp 1954), while *P. dendrobii* produces narrower conidia (Crous & Braun 2003) and *P. peristeriae* is diagnosed by superficial hyphae with solitary conidiophores (Meeboon et al. 2007). Almost all *P. epidendri* caespituli were hypophyllous but not formed through stomata, which may indicate an adaptation for the high solar incidence typical of Brazilian "high altitude grasslands".

Acknowledgments

The authors wish to thank Uwe Braun (Institute of Biology, Martin-Luther-University, Germany) and Jamjan Meeboon (Faculty of Agricultural Technology, Chiang Mai Rajabhat University, Thailand) for reviewing the manuscript. This work is part of an ongoing program of surveying and describing the phytopathogenic and the endophytic mycodiversity associated to the family Orchidaceae in the state of Minas Gerais supported by grants from Fundação de Amparo à Pesquisa do Estado de Minas Gerais - FAPEMIG (project 279/07) and the CNPq fellowship for O.L. Pereira.

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Eremiomyces magnisporus (*Pezizales*), a new species from central Spain

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Abstract — A new species of *Eremiomyces* is described from central Spain. *Eremiomyces magnisporus* is proposed to accommodate a single collection found in July 2010 in a marl-gypsicolous soil at Mount Gurugú, Alcalá de Henares (Spain). The habitat and macro-/micromorphology of the fresh material are described. Molecular analyses of ITS and nLSU DNA support the new species as closely related to but distinct from *Eremiomyces echinulatus* from the Kalahari desert.

Key words — Kalahari truffles, Mediterranean, hypogeous fungi

Introduction

The genus *Eremiomyces* Trappe & Kagan-Zur must be considered as a member of the *Pezizaceae* Dumort. (*Pezizales* J. Schröt.) since it was proposed by Ferdman et al. (2005) to accommodate the molecularly deviant species *Choiromyces echinulatus* Trappe & Marasas [≡ *E. echinulatus* (Trappe & Marasas) Trappe & Kagan-Zur]. *Eremiomyces echinulatus* is a truffle of the African Kalahari, collected in South Africa, Botswana, and Namibia (Trappe et al. 2008, 2010) and, until now, the only known species in this genus.

In the present work, a second *Eremiomyces* species is described from the steppe-like semi-arid hills around Alcalá de Henares, central Spain. These low hills present a marl-gypsum soil covered by introduced *Pinus halepensis* as well as indigenous xerophilous shrubs such as *Macrochloa tenacissima*, a Mediterranean poaceous plant with an Iberian and northern African distribution (Figs. 1–2). Other fungi previously collected in this region include *Battarrea phalloides* (Dicks.) Pers., *Dictyocephalos attenuatus* (Peck) Long & Plunkett, *Galeropsis desertorum* var. *bispora* (Vassilkov) G. Moreno et al., *Gastrosporium simplex* Mattir., *Tulostoma* spp., and *Phaeomyces ibericus* (G. Moreno & Esteve-Rav.) E. Horak.

Figs. 1. Habitat under *Pinus halepensis* with *Macrochloa tenacissima*. 2. Sample collection site.

Materials & methods

Morphology

The type material is preserved in the herbarium of the Dept. of Plant Biology, University of Alcalá de Madrid (AH). Microscopical observations were recorded from tissues mounted in KOH 5%, lactophenol cotton blue, and congo red. Spore measurements do not include superficial structures. Scanning electron microscopy (SEM) images were taken at the University of Alcalá de Henares, using a Zeiss DSM-950 instrument.

DNA extraction, PCR amplification, and DNA sequencing

DNA extraction, PCR amplification, and amplicon purification from type collection AH 38981 was performed as described previously (Alvarado et al. 2010). PCR primers ITS1F and ITS4 (Gardes & Bruns 1993), and ITS4 (White et al. 1990) for the ITS region, and LR1 and LR7 (van Tuinen et al. 1998, Vilgalys & Hester 1990) for the nLSU rDNA region were employed for PCR amplification and sequencing purposes. Sequences were visually inspected for reading errors in MEGA4 (Tamura et al. 2007).

Sequence alignment and phylogenetic analysis

The obtained sequences were aligned with the closest matches from BLAST searches in the public databases. ITS and nLSU sequences came mainly from Læssøe & Hansen (2007) and Perry et al. (2007). Sequences were aligned in MEGA4.0 software using its ClustalW application. The final alignment was assembled manually. Neighbour-joining phylogenetic inference was performed in MEGA4 (pairwise deletion of gaps, 2000 bootstrap replicates). The aligned loci were loaded in PAUP* 4.0b10 (Swofford 2001) and a maximum parsimony phylogenetic tree reconstruction was performed (2000 bootstrap replicates, TBR swapping algorithm, 50 sequence additions per replicate, MULTrees not in effect). Aligned loci were also subjected to MrModeltest 2.3 (Nylander 2004) in PAUP* 4.0b10 to search for the evolutionary models that best fit the data. These models were simulated during the analysis of each locus in MrBayes 3.1 (Ronquist & Huelsenbeck 2003). A Bayesian maximum likelihood (BML) analysis using 4 metropolis-coupled Monte Carlo Markov Chains (MCMC) was performed setting the temperature of the chains to 0.2 and sampling once each 100 generations. The analysis was run until convergence parameters were met in two simultaneous runs, after about 2M generations. A full search for the highest scoring ML trees was also performed in RAxML (Stamatakis 2006) using the standard search algorithm, a thousand bootstrap replications, and allowing an independent evolutionary model for each locus.

Molecular results

The final alignment of the ITS region included 327 of 658 variable sites, 288 of which were also parsimony-informative. In turn, nLSU alignment included 114 parsimony-informative among 151 variable sites from a total 592 bases. Both ITS and nLSU data were best described by the evolutionary model GTR+G+I.

Taxonomy

Eremiomyces magnisporus G. Moreno, P. Alvarado, Manjón & Sanz **sp. nov.**

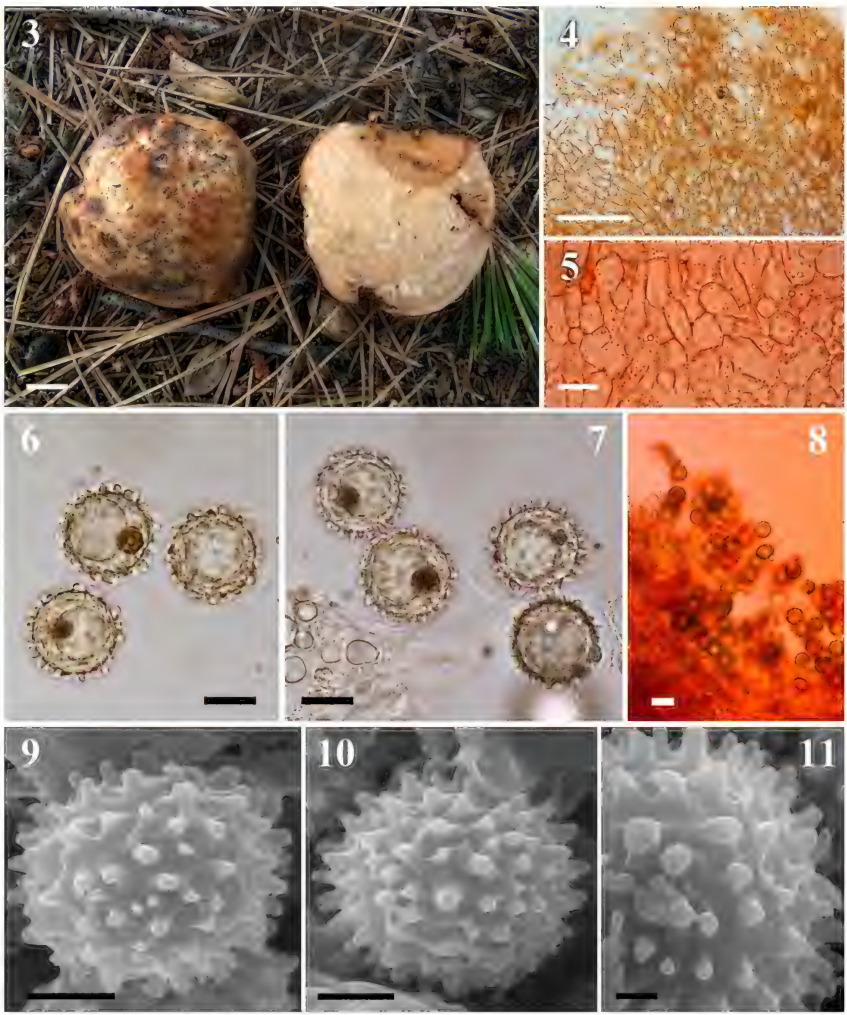
MycoBank MB561665 Figs. 3–11

Ascomata subglobosa, 5 cm longa, peridio pallide brunneo passim atrorufo. Colores iuvenum speciminum permanent post siccationem. Peridium 0.1–0.3 mm crassum, angularis structura, magnis cellulis 25–45 × 5–20 µm composita, praeditum. Gleba griseobrunnea vel griseostraminea numerosis albidis venis incomposite distributis, formantibus varium reticulum. Sporae 14–17 µm sine ornamentis, globosae, hyalinis vel pallide luteolis vacuolis praeditae. Sporarum ornamenta ex obtusis bacillis vel conis dense distributis, in mente revocantibus ornamenta Terfeziae arenariae, *sed perspicue breviora (1.5–2 µm longa, 1 µm lata) et magis dense disposita, efformata. Asci cylindrici difficiles inventu aetate provecta speciminis, videntur una serie dispositi. Paraphyses haud observatae. Odor fortis revocans caseum domesticum.*

Type — Spain, Madrid, Alcalá de Henares, Mount San Juan del Viso, 750 m a.s.l., 10.VII.2010, leg. M.A. Sanz (**Holotype** AH 38981). [GenBank JN032128 (ITS), JN032129 (28S nLSU)].

Etymology — Latin, *magni-* (big) + *-sporus* (spores), referring to the larger size relative to those in *E. echinulatus*.

Ascoma subglobose 5 cm diam., without an obvious attachment point to the substrate, peridium smooth, pale brown with reddish to reddish-black areas where damaged, fresh colours retained after drying. Peridium 0.1–0.3 mm thick, with an angular structure formed by entangled cylindrical hyphae; cells 25–45 × 5–20 µm, becoming more globose toward the interior; cell walls ≤ 1.5 µm, hyaline to slightly yellowish; outermost cell layer 10–40 µm thick, red-brownish vacuolar pigments present; external layer of amorphous debris ≤ 25 µm thick. Gleba beige to dun straw with abundant 0.05–0.4 mm broad whitish veins irregularly distributed forming a variable reticulated mesh with multiple attachment points to the peridium, colour appearing darker where bitten by animals; once dried, small [0.5–1.5 mm diam.] (sub)globose or subglobose spore-containing pockets protrude. Ascospores globose, 14–17 µm (average 15.875, SD 0.545) without ornamentation, hyaline to slightly yellowish, vacuolate; ornamented by densely arranged 1.5–2 × 1 µm blunt-edged rods and cones reminiscent of *Terfezia arenaria* (Moris) Trappe, but clearly shorter and more densely arranged. Asci and paraphyses could not be found due to the advanced maturity of the sample, but spores remained linearly arranged, commonly in groups of 4(–5) spores. Odour strong, similar to cottage cheese.



Figs 3–11. *Eremiomyces magnisporus* (Holotype: AH 38981). 3. Ascoma peridium and gleba. 4. Peridium. 5. Detail of angular peridial cells. 6–7. Spores under LM. 8. Linearly arranged spores. 9–10. Spores under SEM. 11. Detail of spore ornamentation under SEM.
Scale bars: 3 = 1 cm, 4 = 100 m, 5 = 25 µm, 6–7 = 10 µm, 8 = 20 µm, 9–10 = 5 µm, 11 = 2 µm.

Ecology & distribution. *Eremiomyces magnisporus* is a rare species fruiting in steppe-like areas of the marl-gypsum hills surrounding Alcalá de Henares (central Spain).

Comments. A single ascoma was located by the truffle-hunting dog of Miguel Ángel Sanz under *Macrochloa tenacissima* and *Pinus halepensis* in July 2010.

FIGURE 2 shows the exact point of collection under *M. tenacissima*, without any sign of the typical 'burn' associated with other hypogeous fungi. It cannot be concluded which plant species was host of this presumably mycorrhizal fungus. Kalahari truffles have been said to fruit under poaceous species: *Enneapogon cenchroides* (Licht.) C.E. Hubb., *Eragrostis rigidior* Pilg., and *Stipagrostis uniplumis* (Licht.) De Winter (Trappe et al. 2008). Interestingly, *E. echinulatus* was collected in June during the austral winter (Trappe et al. 2010), while *E. magnisporus* appeared in northern hemisphere during the summer.

Eremiomyces magnisporus is characterized by its pale brown peridium with reddish areas that do not blacken after drying, large-sized spores (14–17 µm without ornamentation), and a spore ornamentation formed by very densely arranged blunt-edged hyaline to pale yellow cones. *Eremiomyces echinulatus* is distinguished by a peridium that turns black after drying, dark brown glebal veins, and smaller spores (10–14 µm without ornamentation).

Discussion

Phylogenetic similarities between South African and Mediterranean fungi have been reported before. The recently proposed inocybeoid genus *Tubariomyces* Esteve-Rav. & Matheny is represented in both Spain and Italy and Zambia (Alvarado et al. 2010). *Tubariomyces* species apparently associate with *Cistaceae* Juss. in the Mediterranean and with *Phyllanthaceae* Martinov and *Fabaceae* Lindl. in Zambia.

Macrochloa tenacissima, the putative host plant of *E. magnisporus*, is commonly found in semiarid basic soils of the Mediterranean basin in Turkey, Italy, Spain, Morocco, Algeria, Tunisia, and Libya. In the western Mediterranean, it reaches central Spain and several more scattered spots of northeastern Spain, yet it is not found beyond the high plateaus preceding the Sahara desert to the south and southwest of Morocco. Up to now, *M. tenacissima* has been regarded as an exclusively endomycorrhizal plant, hosting glomalean species such as *Glomus aggregatum* N.C. Schenck & G.S. Sm. and *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler (Roldán-Fajardo 1994). Interestingly, the hypogeous fungus *Gastrosporium simplex* has also been found in association with poaceous species of the *Arrhenathero-Stipetum tenacissimae* association in the hills around Alcalá de Henares (Moreno et al. 1991) as well as with *Festuca ovina* L. (Montecchi & Sarasini 2000).

Of the poaceous genera indicated by Trappe et al. (2010) as the most probable hosts of the Kalahari truffles, those most closely related to *Macrochloa* are *Stipagrostis* Nees, *Eragrostis* Wolf and *Enneapogon* Desv. ex P. Beauv. Although widely distributed, *Eragrostis* is considered typical of subtropical and xerophilous habitats and is common in Spain. Likewise, *Enneapogon* has a split Australian–African distribution, with only *E. desvauxii* P. Beauv. being

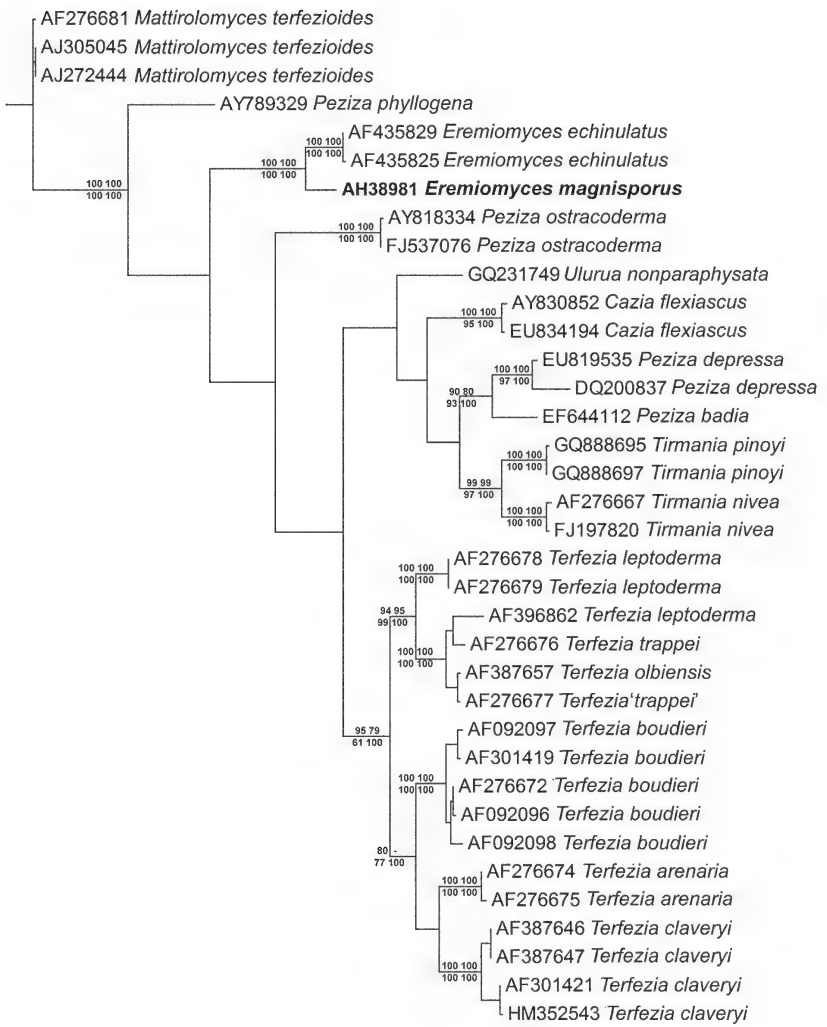


FIG. 12. *Eremiomyces magnisporus* and its closest relatives in the *Pezizaceae*. Highest scoring ITS tree (maximum parsimony, PAUP*). Only nodes supported by three or more of the inference methods are annotated. Values represent (from upper left to lower right): MEGA4 neighbour-joining, PAUP maximum parsimony, RAxML bootstrap proportions, and MrBayes posterior probabilities.

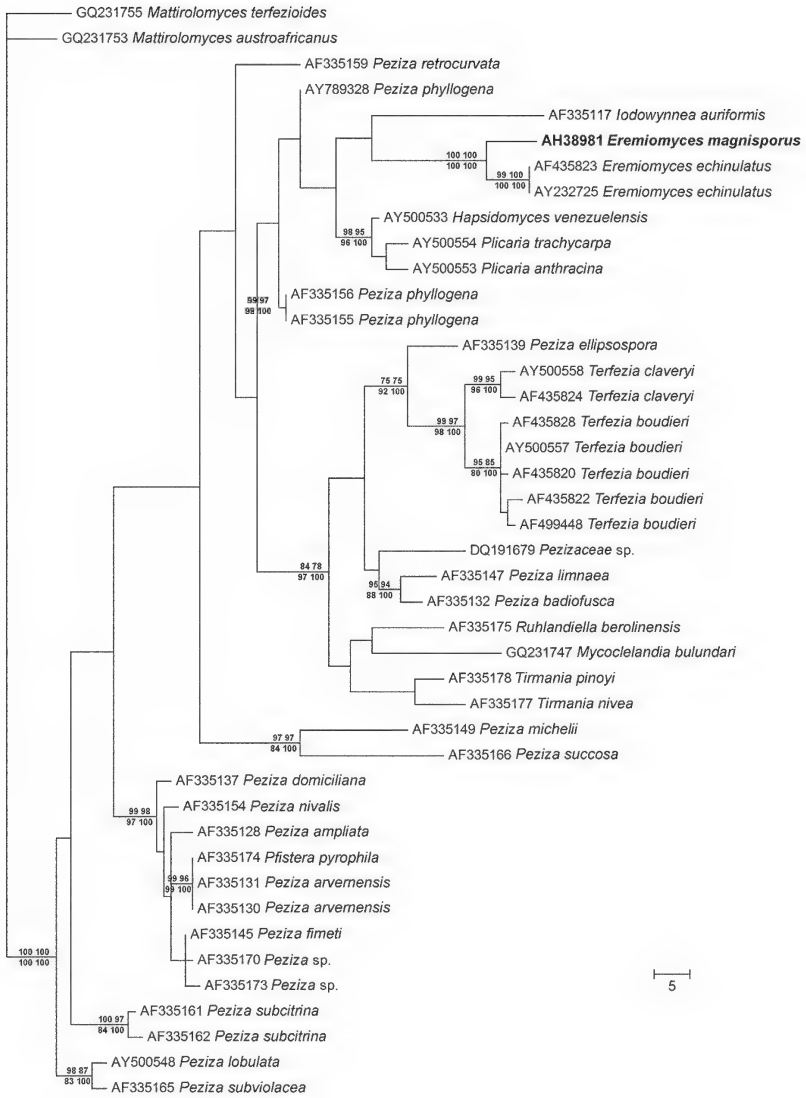


FIG. 13. *Eremiomyces magnisporus* and its closest relatives in the *Pezizaceae*. Highest scoring nLSU rDNA tree (maximum parsimony, PAUP*). Only nodes supported by three or more of the inference methods are annotated. Values represent (from upper left to lower right): MEGA4 neighbour-joining, PAUP maximum parsimony, RAxML bootstrap proportions, and MrBayes posterior probabilities.

widespread. Most African *Enneapogon* species range from south to northeastern Africa, often reaching Arabia and India (Peterson et al. 2010). Only *E. persicus* Boiss. reaches western Mediterranean to southeastern Spain. In turn, *Stipagrostis* is found from southern Africa to Middle East and northwestern India, reaching the western Mediterranean up to Morocco (Le Houérou 2001). It would be interesting to search for this and other hypogeous genera in the distribution areas of the poaceous species mentioned above. More research on *Eremiomyces* and its unexpected plant hosts is clearly needed to shed light on its disjunct geographical distribution

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Peziza paludicola, the correct binomial for *P. udicola* nom. inval.

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Abstract — "*Peziza udicola*", *P. crassipes* Quél., and *P. paludicola* are shown to be conspecific, with *P. paludicola* as the correct name for the species. *Peziza crassipes* var. *kilimanjarensis* is recombined as a variety of *P. paludicola*.

Key words — *Ascomycota*, *Pezizales*, nomenclatural analysis, taxonomy

Introduction

During preparation of a monograph on *Peziza* Fr. —to be published by the Bresadola Mycological Association (AMB, Trento, Italy)— the name *Peziza udicola* Svrček aroused our attention. This name appears only in some identification keys (Hohmeyer et al. 1989, Spooner 2001), generally as a short observation without reference to year and place of publication and sometimes also without author. No information about its date and place of publication was available from the Index of Fungi, IndexFungorum (www.indexfungorum.org), or MycoBank (www.mycobank.org).

A loan request for the holotype from the mycological collections of the National Museum of Prague (PRM), where Svrček's specimens are located, was met with the response that there were no specimens or data relating to this name.

It was moreover necessary to verify the possible conspecificity of "*P. udicola*" with *Aleuria paludicola* Boud. (assumed by Schmid-Heckel 1990) and also with *Peziza crassipes* Quél. (reported by Svrček 1970).

Svrček (1970) provided a detailed description of *P. crassipes* Quél. (free translation from German):

> ...Hymenial surface 250–300 µm tall, tending to brown. Hypothecium narrow, not distinctly delimited from the medulla. Asci 250–300 × 14–22 µm, 8-spored, with apical ring strongly amyloid... Spores (19–)22–24(–25) × (10–)10.5–12.5(–13.5) µm, mostly 21–23 × 11–12 µm, long elliptical to cylindrical-elliptical, round to sharpened at both extremities, homogeneous content, often pale yellow-brown, totally smooth...on decaying twigs, herbaceous stems, completely rotten wood, debris, sometimes on necrotic herbs or directly on bare ground, in humid to swampy places, river banks and near springs, particularly under large herbaceous plants (e.g. *Petasites, Chamaenerion angustifolium*)....

Svrček (1970) also reported:

> ...I cannot exclude the possibility that *Aleuria paludicola* Boudier (1907) and *Peziza crassipes* are conspecific. Boudier's original diagnosis states that this *Aleuria* has saucer-shaped apothecia, spores 23–26 × 12–15 µm and exceptionally wide paraphyses (18–22 µm). Up to now it has been collected by the author only once, on marsh stems in a swamp. Maybe it is an abnormal form of *Peziza crassipes*... Moser (1963) mistakes the two species and unintentionally uses the epithet "*palustris*" for Boudier's "*paludicola*."

In 1970, therefore, Svrček did not make any mention of the earlier homonym, *Peziza crassipes* Wallr. (Wallroth 1833), an inoperculate ascomycete. This earlier homonym makes *P. crassipes* Quél. a nom. illegit. Eleven years later in a checklist of Czech discomycetes, Svrček (1981) listed his 1970 records of *P. crassipes* Quél. under a newly coined name "*P. udicola,*" noting that there was an earlier homonym of Quélet's binomial and referring to the description and illustration in his previous publication: "Svr. 1970: 63 (c. fig.) (*Peziza crassipes* Quél., non Wallroth)". The name "*P. udicola*" was apparently intended as a potential nom. nov. for the illegitimate Quélet binomial, but Svrček failed to meet the requirements for valid publication when he made no full and direct reference to the place of publication of the replaced synonym. As a potential sp. nov., it was also not validly published because of the absence of any Latin description or reference to a previously published Latin description (McNeill et al. 2006: Art. 36.1); it may even be argued that it is a nom. nud. (McNeill et al. 2006: Art. 32.1(d)) despite the direct reference to the previous German description (Svrček 1970) and the indirect reference to the original French description (Quélet 1883).

Specimen loans of the holotype, isotype, or other relevant collections of *P. crassipes* and *Aleuria paludicola* were requested from the fungal collections of the National Museum of Natural History, Paris (PC) but none were found. We have therefore only been able to compare published information.

Table 1 summarizes what each cited author wrote about the three names. Brummelen (1969) noted that microscopic dimensions given by Boudier (1907, 1904–10) contain an error and need to be decreased by 10%. This was taken into account when analysing the spore sizes of *A. paludicola* reported by

Table 1. Comparison of collections of *Peziza crassipes* Quél., and *P. paludicola.*

Taxon Country of origin (Reference)	Apothecium	Hymenium (color)	Receptacle surface	Ascospore size
P. crassipes France (Quélet 1883)	Calyx-shaped, ± slightly cup-shaped, umbilicate, ± stalked, with large base, widely adherent to the substratum	Ochraceous to pale ochraceous-brown, brown-clayey, dirty ochraceous, dark brown	Slightly paler than hymenium, finely brown furfuraceous	(19–)21–23(–26) × 10–13 µm
P. paludicola France (Boudier 1907)	Plane, not cup-shaped, almost pulvinate, shortly stalked, fleshy, thick	Reddish ochraceous-yellow, ochraceous to brown, tawny-brown	Paler than hymenium, finely furfuraceous. Margin not indented	*20.7–23.4 × 10.8–13.5 µm *(acc. to Brummelen 1969)
P. crassipes Czechoslovakia (Svrček 1970)	Slightly cup-shaped / flattened, saucer-shaped to turbinate-cyathiform, sl. depressed, largely adherent, subsessile,	Hazelnut, ochraceous-brown, brown-clayey, yellow-brown	Paler than hymenium, brown-furfuraceous, margin dentate from granulation. Pseudostalk whitish	(19–)21–23(–25) × 11–12(–13) µm
P. paludicola Italy (this paper)	Slightly cup-shaped, depressed, ± umbilicate, sessile to sub stalked, occ. flattened on the bottom	Brownish-ochraceous	Concolorous or slightly paler than hymenium, granulation denser toward margin; edge entire, but appears dentate from granulation	19–22(–24.5) × 12–13 µm

Table 1 continued on next page

Table 1, concluded

Taxon Country of origin (Reference)	Ascus size	Paraphyses	Excipulum structure ME = medullary excipulum EE = Ectal excipulum	Habitat
P. crassipes France (Quélet 1883)	250–300 × 14–18 µm	7–11 µm wide, usually irregular, often enlarged & curved, sometimes with pigment granules	ME: text. globulosa-angularis; EE: similar to ME, but with smaller cells	Wood (also buried), woody humus, sawdust
P. paludicola France (Boudier 1907)	*270–360 × 18–22.5 µm *(acc. to Brummelen 1969)	(16–20 µm wide acc. to Brummelen), apex long club-shaped, thick, weakly coloured	Not reported	On marsh *Carex* leaves or other vegetal residuals in swampy areas
P. crassipes Czechoslovakia (Svrček 1970)	250–300 × 14–22 µm	7–11 µm, clavate, irregularly wrinkled	Subhymenium: not distinguishable. ME: text. globulosa-angularis, cells 60–90 µm. EE: similar to ME, cells smaller, 18–40(–60) µm.	Marsh wood (also buried), sawdust, twigs, herbaceous stems, swampy ground or very humid places with large herbaceous plants
P. paludicola Italy (this paper)	250-280 × 15–18 µm	Cylindrical medially, 1–2 basal cells swelling to 4–5 µm, apex irregular and ≤ 10 µm, simple or forked in the lower part	Subhymenium: text. globulosa or gl.-angularis, cells 10–30 µm. ME: similar text., cells ≤100 µm mixed with intricate hyphae. EE: text. similar to ME, cells 10–15 µm.	Degraded deciduous wood; loam rich in woody herbaceous residuals

Boudier. The corrected dimensions of the spores in question here are 20.7–23.4 × 10.8–13.5 µm. Comparing these data with those obtained from our analyses on "*P. udicola*" makes clear that the various characteristics are very similar and that small differences may be considered physiological variations of different individuals. We believe they are not enough to justify treating the names as separate species.

Materials & methods

Macro- and microscopic examinations were carried out on both fresh and dried specimens (rehydrated in water or KOH 5%), with water as a mounting medium and Melzer's reagent to observe the iodine reaction of the asci, using an Optika optical microscope (BK 1301 model), with 40× or 100× (immersion oil) objectives. Spore dimensions were obtained by measuring of 50 mature spores. Collections were placed in the fungal reference collection of L'Aquila (HMA), the Royal Botanic Gardens, Kew K(M), and the private herbaria collections of F. Padovan (FP) and G. Medardi (GM).

Taxonomy

Peziza paludicola (Boud.) Sacc. & Traverso, Syll. Fung. 20: 315, 1911. FIG. 1

≡ *Aleuria paludicola* Boud., Hist. Classific. Discomyc. Europe: 46, 1907

= *Peziza crassipes* Quél., C.R. Ass. Franç. Av. Sci. (La Rochelle 1882) 11: 405, 1883, nom. illegit., non Wallr. 1833.

"*Peziza udicola*" Svrček, Česká Mykologie 35(2): 72, 1981 nom. inval., ICBN [Vienna] Art. 36.1.

TYPIFICATION: **Lectotype designated here**, FRANCE, "sur les feuilles pourries des *Carex*, Juillet, dans les marais. Montmorency", *Aleuria paludicola* coloured plate, Boudier, Icon. Mycol., Série 5(24): no. 469 [Tom. 2: pl. 269], 1909. **Epitype designated here**, ITALY, Trentino-Alto Adige, Belluno, shore of the river Piave, 10/05/86, leg. e det. F. Padovan [K(M) 169757].

APOTHECIA up to 20 mm diam., slightly cup-shaped, sessile to sub-stalked. Hymenial surface smooth, slightly depressed to more or less umbilicate, sometimes flattened, brownish-ochraceous. RECEPTACLE SURFACE concolorous or slightly paler than the hymenium and with brown granules, more dense toward the margin; margin regular, appearing dentate because of the granulation, but actually entire. FLESH fragile and waxy, greyish-ochraceous. ASCOSPORES 19–22(–24.5) × 12–13 µm, elliptical, smooth, hyaline, without oil drops, uniseriate in the ascus. ASCI 250–280(–300) × 15–18 µm, sub-cylindrical. PARAPHYSES sub-cylindrical and 4–5 µm wide at the base (often with 1–2 basal enlarged cells), dilated and irregularly shaped in the upper part, up to 10 µm, simple or forked at the base, septate. HYMENIUM up to 300 µm thick. SUBHYMENIUM 40–50 µm thick; textura globulosa-angularis, composed of rounded or slightly angular cells, 10–30 µm diam. MEDULLARY EXCIPULUM

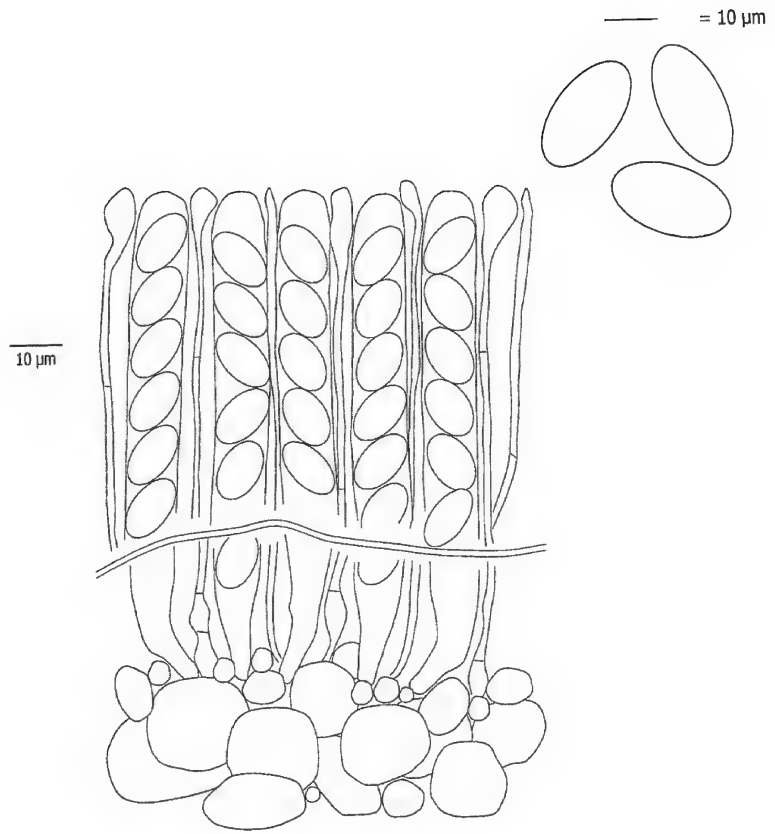

Fig. 1. *Peziza paludicola* (Epitype, K(M) 169757). A. Uppermost zone of the hymenium (tips of paraphyses and asci with ascospores). B. Released ascospores.

about 500 µm thick; textura globulosa, composed of rounded cells up to 100 µm diam., mixed with obvious intricate hyphae, 15–20 µm wide. Ectal excipulum 20–30 µm thick; textura angularis, composed of angular or sub-angular cells, about 10–15 µm diam.

Habitat: in small groups on degraded wood of deciduous trees.

Additional material examined: **ITALY**, **Abruzzo**, L'Aquila, Castellafiume, 09/11/84, leg. e det. not declared, sub *P. ampliata*, (HMA 4129); **Lombardy**, Brescia, Breno, tableland of Gaver, 21/08/04, leg. e det. G. Medardi, (in herb. priv. GM); Ponte di Legno, P. so Gavia, on loam and humid woody herbaceous remnants, at 2800 m of altitude, 06/08/06, leg. e det. G. Medardi, (in herb. priv. GM).

Table 2. Comparison of *Peziza paludicola* with similar species.

Taxon Country of origin (Reference)	Hymenium color	Ascospore size	Excipulum structure ME = medullary excipulum EE = Ectal excipulum	Habitat
Peziza ampliata Italy (this paper)	Whitish-brown or pale brown	20–22 × 12–14 µm	Subhymenium: text. globulosa-angularis. ME: 1 layer of text. globulosa with very large cells (140–220(–240) × 60–80 µm) EE: text. globulosa-angularis, cells 15–35(–45) µm diam.	Herbaceous and woody remnants
Peziza boudieri Italy (this paper)	Brown-yellow-olivaceous with orange reflexes	(18–)20–21 × 7–9 µm	Subhymenium: text. globulosa-angularis. ME:: 1 layer of text. intricata. EE: text. globulosa	Ground
Peziza hortensis Italy (this paper)	Tawny-brown with yellowish reflexes	18–20(–22) × 10–11 µm	Subhymenium: text. globulosa-angularis. ME: 3 layers (1° text. globulosa, 2° text. intricata, 3° text. globulosa-angularis). EE: text. intricata	Ground and cultivation loam
Peziza paludicola Italy (this paper)	Brownish-ochraceous	19–22(–24.5) × 12–13 µm	Subhymenium: text. globulosa-angularis, cells 10–30 µm diam. ME: 1 layer of text. globulosa, cells up to 100 µm diam. EE: text. angularis, cells 10–15 µm diam.	Deciduous wood
Peziza sciophila Italy (Medardi 2007)	Hazelnut-grey-ochraceous with weak olivaceous or violaceous-purple tinges	18–21 × 10–12 µm	Subhymenium: not distinguishable. ME: 2 layer (1° text. intricata, 2° text. intricata & irregularly globose cells. EE: text. globulosa	*Picea* wood litter / residuals; more rarely on *Alnus* remnants

Peziza paludicola var. ***kilimanjarensis*** (J. Moravec) Cacialli, Lantieri & Medardi, **comb. nov.**

MycoBank MB 561192

≡ *Peziza crassipes* var. *kilimanjarensis* J. Moravec, Česká Mykologie 32(2): 77. 1978.

The Moravec (1978) variety required transfer from the illegitimate species, *P. crassipes* Quél., to its legitimate synonym *P. paludicola*.

Discussion

Peziza paludicola can be distinguished from similar species by the following characteristics: apothecia with monotonous colours; the outside showing a dark granulation more evident at the margin; smooth and relatively large spores; habitat generally related to decaying deciduous wood. Similar species are *Peziza boudieri* (Cooke) Donadini, *Peziza hortensis* P. Crouan & H. Crouan, and *Peziza sciophila* Medardi. These can be differentiated by the anatomy of the excipular tissues and by the habitat. *Peziza ampliata* Pers. is distinctive in both the excipular architecture and the position of the layers and the dimensions of the cells; the substratum is also at times important to separate these fungi. Table 2 summarizes the main characteristics of these species.

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Two new species of *Corynespora* from northeastern Uttar Pradesh, India

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Abstract — Two *Corynespora* species collected from northeastern Uttar Pradesh, India, are described and illustrated: *C. carrisae* sp. nov. from living leaves of *Carissa spinarum* (*Apocynaceae*) and *C. peristrophicola* sp. nov. from living leaves of *Peristrophe bicalyculata* (*Acanthaceae*).

Key words — biodiversity, hyphomycetes, foliar diseases, phytopathogenic fungi, taxonomy

Introduction

Indian researchers have added several novel species to the anamorphic genus *Corynespora* during the past few years (Meenu et al. 1997, Meenu & Kamal 1998, Sharma et al. 2002, Jain et al. 2002, Pal et al. 2007, Singh et al. 2007, Kumar et al. 2008). During our recent survey, we encountered several plant species exhibiting leaf blights. Upon critical examination and a thorough survey of the literature, we found that two blights were caused by two new *Corynespora* species: *C. carrisae* on *Carissa spinarum* L. (*Apocynaceae*) and *C. peristrophicola* on *Peristrophe bicalyculata* (Retz.) Nees. (*Acanthaceae*). These taxa are described and illustrated below.

Materials & methods

Infected leaf samples from different parts of northeastern Uttar Pradesh (U.P.) were placed in separate polythene bags and taken to the laboratory. Suitable mounts of surface scrapings and free-hand cut sections were prepared from infected portions of the leaf samples. The material was mounted in a cotton-blue lactophenol mixture on microscope slides. The slides were examined, the specimens were measured and camera lucida drawings were made. Morphotaxonomic determinations were made with the help of current literature and available resident expertise. Holotypes have been deposited in HCIO (Herbarium Cryptogamiae Indiae Orientalis), Indian Agricultural Research

Institute, New Delhi; isotypes were retained in the herbarium of the Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, for further reference.

Taxonomy

Corynespora carrisae R. Singh & Kamal, **sp. nov.** FIG. 1

MycoBank MB 519052

Maculae amphigenae, circulares vel subcirculares, 3–5 mm in diam., brunneae vel atrae. Coloniae amphiphyllae, effusae, brunneae. Mycelium internum, tenuitnicatum, glabrum, ex hyphis ramosis, olivaceo brunneis vel brunneis. Stromata nulla. Conidiophora singularia, macronematosa, mononematosa, erecta vel procumbenta, recta vel flexuosa, nonramosa, cylindrica, glabra, crassitunicata, 4–8-septata et 1–15 successivas proliferationes, 250–675 × 4–10 μm, cellula basalis inflatis, brunneis vel atro brunneis. Cellulae conidiogenae in conidiophoris integratae, terminales, monotreticae, cicatrices non incrassates. Conidia acrogenaes, solitaria, simplicia, non ramosa, tenui tunicata, glabra, recta vel curvata, obclavato-cylindrica, 4–17-distoseptata, 75–242 × 6–14 μm, ad apicem obtusa vel rotundata, olivacea vel luteo brunnea, hilo in crassato, germinato conidium notatum.

Type: On living leaves of *Carissa spinarum* (*Apocynaceae*), Kusumhi forest, Gorakhpur (U.P.), India, 19 September 2007, coll. Raghvendra Singh, HCIO No. 48276 (**holotype**), GPU Herb No. KR-10 (**isotype**).

Etymology: the epithet is derived from the genus name of the host.

Infection spots amphigenous, circular to subcircular, 3–5 mm in diameter, brown to black. Colonies amphiphyllous, effuse, brown. Mycelium internal, thin-walled, smooth, branched, olivaceous brown to brown. Stromata absent. Conidiophores arising singly, macronematous, mononematous, erect to procumbent, straight to flexuous, unbranched, cylindrical, smooth, thick-walled, 4–18-septate and 1–15 successive cylindrical proliferations, 250–675 × 4–10 μm, basal cell swollen, brown to dark brown. Conidiogenous cells integrated, terminal, monotretic, scars unthickened. Conidia acrogenous, solitary, simple, unbranched, thin-walled, smooth, straight to slightly curved to obclavate-cylindrical, 4–17-distoseptate, 75–242 × 6–14 μm, apex obtuse to rounded, olivaceous to very light brown, hilum thickened, germinating conidia present.

Remarks— Only one *Corynespora* species, *C. alstoniae* Meenu et al. (Meenu et al. 1997), has been described on *Apocynaceae*. Compared with the present collection, *C. alstoniae* has shorter and broader conidiophores (121–473.5 × 6.0–13.5 μm) and conidia (48.5–154 × 8.5–21.5 μm). The hilum in *C. carrisae* is thickened while in *C. alstoniae* it is unthickened.

Unbranched to branched and longer conidiophores (110–850 × 4–11 μm) easily distinguish *C. carrisae* from *C. cassiicola* (Berk. & M.A. Curtis) C.T. Wei (Wei 1950), which has unbranched, shorter conidiophores. Its 4–17-distoseptate longer thinner conidia also contrast with the 4–20-distoseptate shorter thicker (40–220 × 9–22 μm) conidia in *C. cassiicola.*

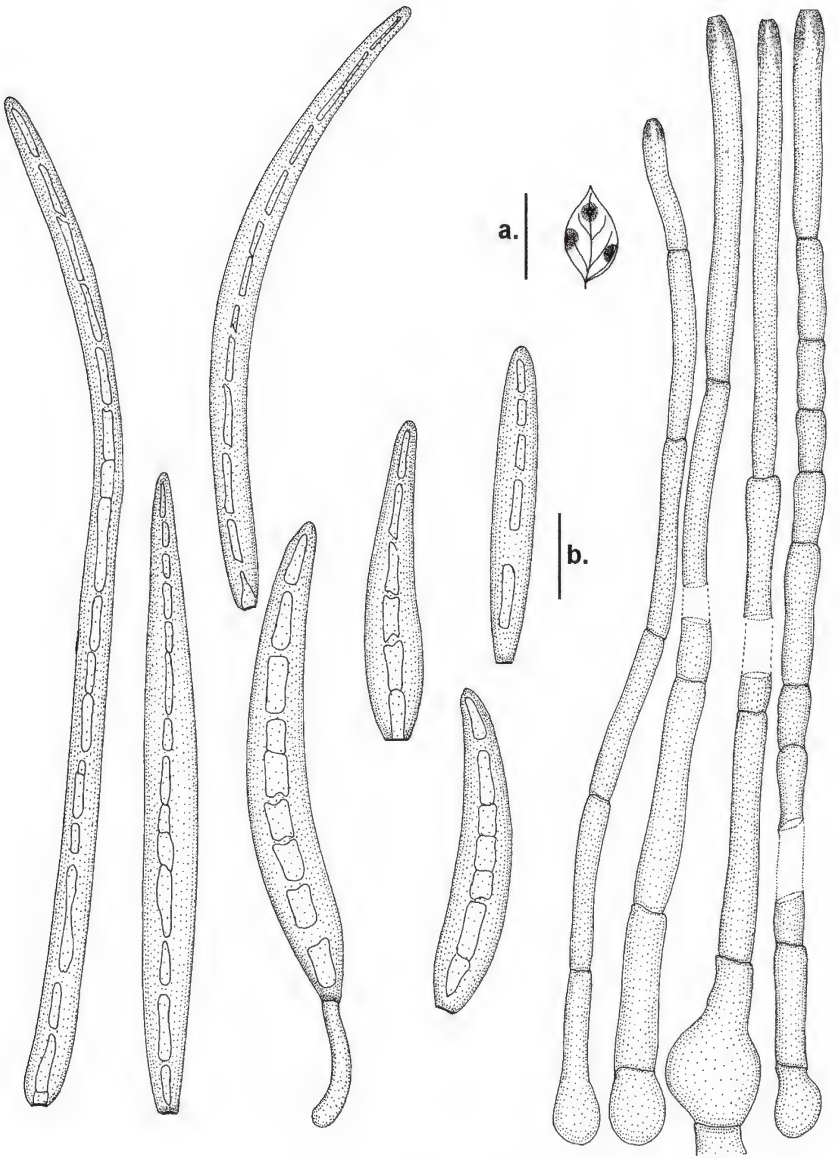

FIG. 1. *Corynespora carrisae.*
a: symptoms; b: conidia, germinating conidia, and conidiophores.
(Scale bars: a = 20 mm, b = 20 μm).

The new species appears to be most similar to *C. euphorbiacearum* Meenu et al. (Meenu et al. 1997). *Corynespora carrisae* has more septa (4–18), more successive cylindrical proliferations and longer conidiophores compared with *C. euphorbiacearum* with 3–7-septate, shorter conidiophores (100–358 × 6–8 μm), and thickened noncatenate and narrower conidia separate *C. carrisae* from *C. euphorbiacearum* conidia that are unthickened, solitary to catenate, and broader (59–235 × 11.5–22.5 μm).

One other possible close relative is *C. gigaspora* (Berk. & Broome) M.B. Ellis (Ellis 1957), which produces conidia that are longer and broader (100–270 × 19–28 μm) with more distosepta (9–52).

Corynespora peristrophicola R. Singh & Kamal, **sp. nov.** FIG. 2

MycoBank MB 519051

Maculae epigenae, circulares vel subcirculares/irregulares, 1–18 mm in diam., brunneae vel atrae. Coloniae epiphyllae, effusae, grisae. Mycelium internum, tenui tunicatum, glabrum, ex hyphis ramosis, olivaceo-brunneis vel brunneis. Stromata nulla. Conidiophora superficialia, ex hyphis oriunda singulata vel 2–3-fasciculata, macronematosa, mononematosa, cylindrica, recta vel procumbenta, erecta vel flexuosa, non ramosa, glabra, crassitunicata, 5–18-septata et 1–3 successivas proliferationes, medio brunnea, 120–325 × 5–10 μm, cellula basalis inflatis. Cellulae conidiogenae in conidiophoris incorporatae, terminales, monotreticae, cicatrices incrassatae. Conidia acrogena, sicca, solitaria, simplicia, non ramosa, tenui tunicata, glabra, recta vel leniter curvata, obclavata vel obclavato-cylindricata, 5–12-distoseptata cum 0–1 angulis distoseptis simulatibus, 60–135 μm longa et 5–16 μm lata, apicem obtusa vel rotundata, olivacea vel luteo-brunnea, hilo crassato praedita.

Type: On living leaves of *Peristrophe bicalyculata* (*Acanthaceae*), Gorakhpur University campus, Gorakhpur (U.P.), India, 2 December 2007, coll. Raghvendra Singh, HCIO No. 48278 (**holotype**), GPU Herb No. KR-12 (**isotype**).

Etymology: the epithet is derived from the genus name of the host.

Infection spots epigenous, circular to subcircular/irregular, 1–18 mm in diameter, brown to blackish. Colonies epiphyllous, effuse, grayish. Mycelium internal, thin-walled, smooth, branched, olivaceous to brown. Stromata absent. Conidiophores arising singly as lateral branches from superficial hyphae, solitary or in fascicle of 2–3, macronematous, mononematous, cylindrical, erect to procumbent, straight to flexuous, unbranched, smooth, thick-walled, 5–18-septate with 1–3 successive cylindrical proliferations, mid brown, 120–325 μm long and 5–10 μm wide, basal cell swollen. Conidiogenous cells integrated, terminal, monotretic, scars unthickened. Conidia acrogenous, dry, solitary, simple, unbranched, thin-walled, smooth, straight to slightly curved, usually obclavate to obclavate-cylindrical, 5–12- distoseptate with 0–1 transverse band-like distosepta, 60–135 μm long and 5–16 μm wide, apex obtuse to rounded, olivaceous to very light brown, hilum thickened.

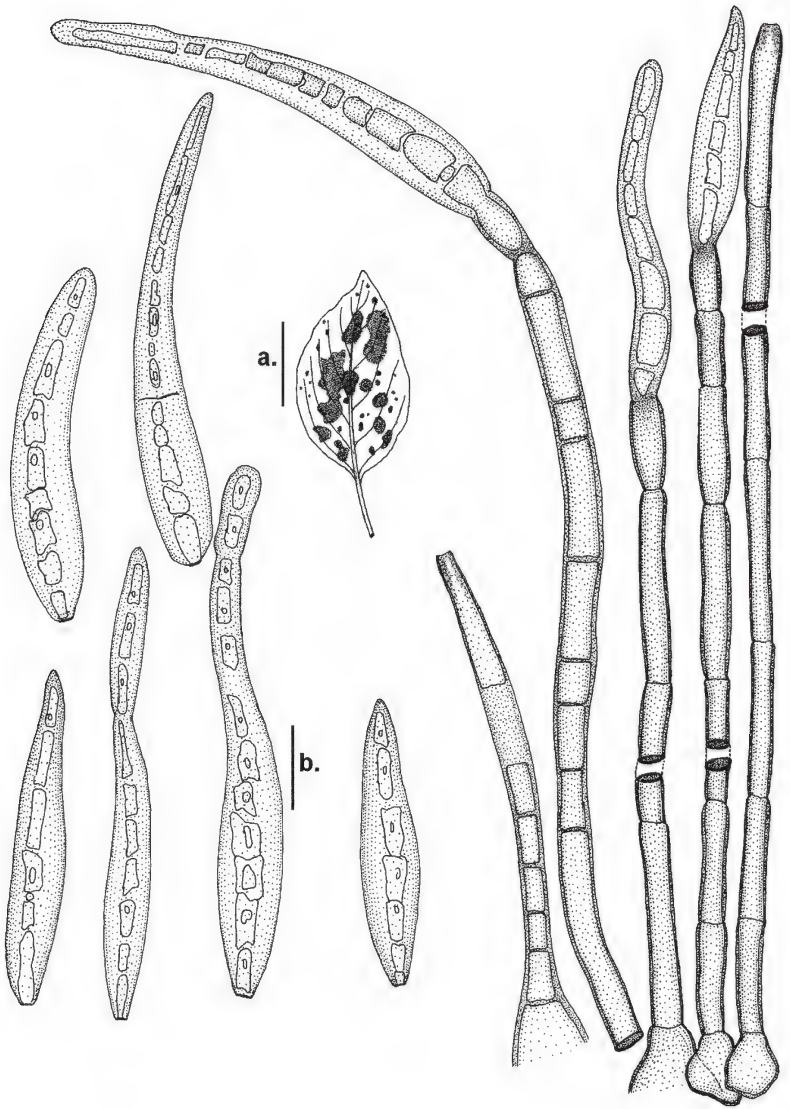

Fig. 2. *Corynespora peristrophicola.*
a: symptoms; b: conidia and conidiophores.
(Scale bars: a = 20 mm, b = 20 μm).

Remarks— Only *C. barleriicola* N. Sharma et al. (Sharma et al. 2002) has is known to occur on members of *Acanthaceae*. *Corynespora peristrophicola* has shorter unbranched 5–18-septate conidiophores while those in *C. barleriicola* are branched, 3–7-septate, and longer (253–479 × 7–9 µm). Furthermore, *C. barleriicola* has longer conidia (41–246 × 10–18.5 µm), which are 3–17-distoseptate with 0–5 distinct transverse band-like distosepta, and its hila are unthickened.

The conidia of *C. peristrophicola* appear most similar to those of *C. siwalika* (Subram.) M.B. Ellis (Ellis 1961: 88–140 × 15–20 µm), *C. leptoderridicola* M.B. Ellis (Ellis 1957: 70–120 × 14–17 µm), and *C. combreti* M.B. Ellis (Ellis 1963b: 40–122 × 8–11 µm) but lack the rostrate morphology typical of the other species. The 5–12 conidial distosepta in *C. peristrophicola* are slightly more numerous than in *C. combreti* (4–10-distosepta) and less numerous than in *C. leptoderridicola* (6–16-distosepta) and *C. siwalika* (9–19-distosepta).

The new species also resembles *C. alstoniae* (Meenu et al. 1997), which, however, has longer broader conidiophores (121–473.5 × 6–13.5 µm) and broader, catenate unthickened conidia (48.5–154 × 8.5–21.5 µm).

Other possible closely related species are separated by number of distosepta: *C. bdellomorpha* M.B. Ellis (Ellis 1963a) with 12–19 and *C. eranthemi* J.M. Yen & Lim (Yen & Lim 1980) with 5–25.

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New lichenicolous fungi records for Kyrgyzstan, Uzbekistan, and Ukraine

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Abstract — *Gemmaspora lecanorae* and *Rosellinula haplospora* are newly reported for Uzbekistan (Asia), *Rosellinula frustulosae* for Kyrgyzstan (Asia), and *Muellerella ventosicola* and *Weddellomyces heterochrous* for Ukraine (Europe). The different ascospore sizes reported for *W. heterochrous* are also briefly discussed.

Key words — *Ascomycota*, cephalothecioid plates, lichens

Introduction

More than 1500 species of lichenicolous fungi had been described by 2003 (Lawrey & Diederich 2003), and 3000–4000 are estimated worldwide (Hawksworth 2001). Lichenicolous fungi have been one of the least explored fungi because of the inadequate literature and a confused taxonomy based largely on nineteenth century concepts (Hawksworth 1983). But with the recent publication of new keys (Foucard 2001, Nash et al. 2004, Halıcı 2008, Ihlen & Wedin 2008) and checklists (e.g. Kocourková 2000, Diederich & Sérusiaux 2000, Scholz 2000, Faltynowicz 2003, Hawksworth 2003), these organisms have started to receive more attention. Here, we report new records of lichenicolous fungi for Ukraine (Europe) and Kyrgyzstan and Uzbekistan (Asia).

Material & methods

Specimens reported are deposited in KW (Kyiv, Ukraine) or KHER (Kherson, Ukraine). Specimens were examined with a Leica DM 1000 research microscope. Microphotographs were taken with a Leica DFC 420 digital microscope camera with c-mount interface and with a 5 megapixel CCD. Sections were prepared by hand and examined in Merck Lugol's iodine (I) and water. Ascospores were measured in water.

Ascospores and asci measurements are given by the arithmetic mean flanked by the mean ± standard deviation and parenthetical minimum and maximum values. All measurements and ratios include the halo in ascospores. The length/breadth ascospore ratio is indicated as l/b.

Taxonomy

Gemmaspora lecanorae (Werner) D. Hawksw. & Halıcı

A detailed description is provided by Hawksworth & Halıcı (2007).

The species is lichenicolous on the thallus of *Aspicilia* species and evidently commensalistic, as no damage or discoloration was observed in the host thallus. Perithecia are black, >300 µm wide, semi-immersed to almost superficial. Asci are 8-spored and ascospores (0–)1-septate, with very thick cell walls, which are 2-layered, and very broadly ellipsoid to globose, (12–)12.5–13–14 × 8.5–9 µm with l/b ratio (1.35–)1.4–1.5–1.6(–1.65).

Gemmaspora lecanorae was previously known from just two localities: Syria, on the thallus of *Aspicilia* cf. *farinosa*, and Turkey, on the thallus of *Circinaria calcarea* (L.) A. Nordin & al. (Hawksworth & Halıcı 2007). New to Uzbekistan on *A. thjanschanica* Oxner.

UZBEKISTAN. Tashkent region, Western Tian'-Shan', Velykyi Chymgan Mountain, 1800 m alt., granite outcrop, on the thallus of *Aspicilia thjanschanica* with *Rosellinula haplospora*. A. Lazarenko, 28.09.1929 (KW 23638, isolectotype of *Aspicilia thjanschanica*).

Muellerella ventosicola (Mudd) D. Hawksw.

A detailed description (as *M. pygmaea* var. *ventosicola*) is provided by Triebel (1989) and Triebel & Kainz (2004).

The species is lichenicolous on the thallus of on a wide range of crustose saxicolous lichens including *Rhizocarpon*, *Ophioparma,* and *Protoparmelia* (Triebel 1989, Ihlen & Wedin 2008, Halıcı 2008) and apparently commensalistic, as no damage or discolouration was observed in the host thallus. Ascomata perithecioid, black, semi-immersed, 250–300 µm. Asci (20–)50-spored, 50–55 × 15 µm. Ascospores brown, thick-walled, 1-septate, 6–7.5–9(–10) × 4–4.5–5 (–6) µm, l/b ratio (1.3–)1.4–1.6–1.8(–2).

Muellerella ventosicola grows on *Rhizocarpon geographicum* (L.) DC. and *R. grande* (Flörke ex Flot.) Arnold. New to Ukraine

UKRAINE. Zakarpattia (Transcarpathian) Region, Rakhiv district, Svydovets, Blyzhnytsia Mountain, 1500 m alt., on the thallus of *Rhizocarpon grande*, M. Makarevych, 11.07.1947 (KW 27534). Zaporizhzha Region, Chernigyvskyi district, Novopoltavka Village, Synia outcrop, on the granite, on the thallus of *R. geographicum*, O. Khodosovtsev, 02.10.2007 (KHER 1469).

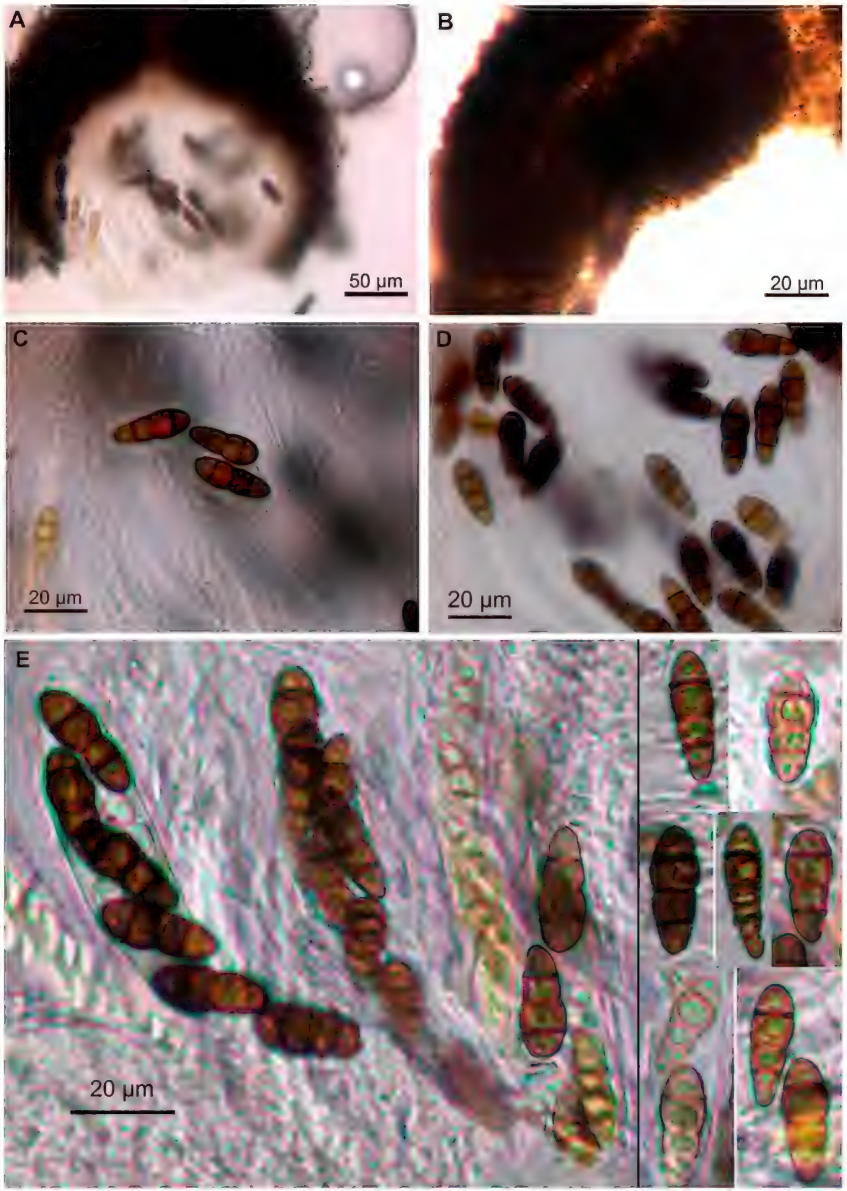

Fig. 1. *Weddellomyces heterochrous*. A, Perithecium; B, Cephalothecioid plates; C, Ascospores and pseudoparaphyses; D, Ascospores; E, 6-spored and 8-spored asci; F, Ascospores.

Rosellinula haplospora (Th. Fr. & Almq.) R. Sant.

A detailed description is provided by Hafellner (1985).

The species has previously been reported as lichenicolous on the thalli of *Circinaria caesiocinerea* (Nyl. ex Malbr.) A. Nordin et al., *Aspicilia cinerea* (L.) Körb., *A. intermutans* (Nyl.) Arnold, and *Bellemerea alpina* (Sommerf.) Clauzade & Cl. Roux (Hafellner 1985; Ihlen & Wedin 2008; Halıcı 2008). It is evidently commensalistic, as no damage or discolouration was observed in the host thallus of our collections, where it grew together with *Gemmaspora lecanorae* and *Lichenostigma elongatum* Nav.-Ros. & Hafellner (on *Aspicilia* spp.). Ascomata perithecia 200–230 µm, 20–50 spores per ascus, (5–)6–6.5–7.5(–8) × (4–)4.5–5–5.5(–6) µm, with l/b ratio (1–)1.1–1.3–1.5(–2).

New to Uzbekistan on *Aspicilia thjanschanica* and *Aspicilia* species in our report.

UZBEKISTAN. Tashkent region, Western Tian'-Shan', Velykyi Chymgan Mountain, 1800 m alt., granite outcrop, on the thalli of *Aspicilia thjanschanica* and *Aspicilia* sp. A. Lazarenko, 28.09.1929 (KW 23638, isolectotype of *Aspicilia thjanschanica*).

Rosellinula frustulosae (Vouaux) R. Sant.

A detailed description is provided by Hafellner (1985).

The species is lichenicolous on the thallus of *Lecanora frustulosa* and *L. argopholis* and evidently commensalistic, as no damage or discolouration was observed in the host thallus. Perithecia, 220–280 µm, c. 100 spores per asci. Spores (4.5–)5.5–6–7(–7.5) × (3.5–)4–4.5–5(–6) µm, with l/b ratio (1–)1.1–1.3–1.5(–1.8).

Rosellinula frustulosae is specific to *Lecanora frustulosa* (Dicks.) Ach. and *L. argopholis* (Ach.) Ach. (Hafellner 1985). New to Kyrgyzstan on *L. argopholis*. Although we have no fresh collections from Ukraine, the holotype was collected from a *L. frustulosa* thallus by K. Mereschkowsky near Simpheropol in Crimea (Hafellner 1985). This is still the only known Ukrainian locality of this species, but it has not yet been reported in Ukrainian check-lists (Kondratyuk at al. 1998, Kondratyuk at al. 2010).

KYRGYZSTAN. Issyk-Kul', Pokrovka, Kyrgyz Station, Institute of Geography AS USSR, in the river valley, on the thallus of *Lecanora argopholis*, on granite. Sobol', 15.07.1947 (KW 3898 – specimen is kept under name "*Rhizocarpon geographicum*").

Weddellomyces heterochrous Nav.-Ros. & Cl. Roux FIGURE 1

A detailed description is provided by Navarro-Rosinés & Roux (1995).

The species is lichenicolous on the areoles of *Aspicilia* and *Circinaria* species and causes a slight bleaching in the infected parts. It is weakly parasitic. The following description is based on our collection on *Aspicilia contorta* subsp. *hoffmanniana* S. Ekman & Fröberg ex R. Sant.

Asci cylindrical or largely cylindrical clavate, (110–)111–*113*–115(–118) × (18–)19.5–*20.5*–21.5(–25) µm (n = 20), 4–6–8-spored, wall gradually thickened towards the apex, I–, ocular chamber present. Ascospores 3-septate, brown, terminal cells concolorous with central cells, surface smooth, halonate, halo ~1 µm, slightly constricted at the septa, but sometimes strongly constricted in the middle septa, heteropolar, uniseriate or irregularly biseriate in the asci, (21–)23–*25*–27(–28) × (8–)9–*9.5*–10(–11) µm (n = 40), l/b = (2.2–)2.4–*2.6*–2.8(–3.1) The ascospore sizes in our data and cited in the literature seem very variable. In the original description, based on a single type on *Circinaria calcarea,* the ascospores (primarily 3-septate) measure (25–)26.5–*30.4*–35.5(–38) × (10–)11–*12*–13.5(–14.5) µm (Navarro-Rosinés & Roux 1995). In a Turkish collection on an *Aspicilia* species, the ascospores measured (32–)36–40 × 11–13 µm (Halıcı et al. 2007). More collections are needed to reassess the ascospore size and host variations and to determine whether more than a single taxon exists.

New to Ukraine.

UKRAINE. Luhans'k Region, Pereval's'k district, steppe slope between Mikhailivka and Troits'ke Villages above Isakiivs'ke water storage, on thallus of *Aspicilia contorta* subsp. *hoffmanniana* on limestone outcrop, O. Nadyeina 09.04.2007 (KW 68135).

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New records of *Agaricales* from Atlantic Forest fragments of Pernambuco, Northeast Brazil

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Abstract — Some interesting fungi were collected during recent expeditions to Atlantic Forest fragments. *Entoloma aripoanum* is recorded for the first time from Brazil. *Crepidotus flavus*, *E. tucuchense*, and *Lepiota erinana* are new records from Brazil's northeast region, and *Trogia cantharelloides* is new from Pernambuco State. Drawings and descriptions of the species are provided.

Key words — *Agaricaceae*, *Crepidotaceae*, *Entolomataceae*, neotropics, taxonomy

Introduction

The Atlantic Forest, which is a priority area for conservation (Myers et al. 2000), has been drastically diminished by human activity since the beginning of Portuguese colonization (Kimmel et al. 2008, Trindade et al. 2008). Only 11–16% remains of the original forest (Ribeiro et al 2009). Research on fungal diversity is urgently needed in this threatened biome.

In this continuation of previous reports on agarics from Atlantic Forest fragments of Pernambuco (Wartchow 2006, Wartchow & Maia 2007, Wartchow et al. 2007a,b, 2008a,b), we present new records of *Crepidotus* (Fr.) Staude, *Entoloma* (Fr.) P. Kumm., *Lepiota* (Pers.) Gray, and *Trogia* Fr.

Material & methods

Collections were undertaken at the Usina São José (7°50′20″S 35°00′10″W), an area covered by variously sized (12–380 ha) Atlantic Forest fragments among sugar cane fields (Alves-Araújo et al. 2008, Trindade et al. 2008). These preserved fragments shelter 650 species (379 genera, 105 families) of trees, with *Fabaceae*, *Poaceae*, *Cyperaceae*,

Asteraceae, *Euphorbiaceae*, *Myrtaceae*, *Rubiaceae*, *Melastomataceae*, *Araceae*, *Malvaceae*, *Apocynaceae*, *Sapindaceae,* and *Sapotaceae* as the most diverse (Alves-Araújo et al. 2008).

The usual methodology for studying agarics was followed (Singer 1986). Color codes and names follow Maerz & Paul (1950; 'M&P'); x = average dimension. Exsiccates are deposited at URM Herbarium (Thiers 2011).

Taxonomy

Crepidotus flavus Capelari, Mycotaxon 115: 146. 2011. FIG. 1

PILEUS 5–14 mm, uniformly sulfur yellow (M&P 10J1 "Sulphur Y, Citrus"), convex with incurved margin in young specimens, then more or less straight, moderately tomentose/pubescent when young, smooth and glabrous in age; context thin, fleshy, cream to pale yellow, unchanging. LAMELLAE radiating from an attachment point, narrow (<0.5 mm broad) concolorous with pileus, then ferruginous in older basidiomata, crowded, with lamellulae. STIPE absent or very rudimentary.

BASIDIOSPORES 6.5–8.5 × 6–8 µm, x = 7.6 × 7.2 µm, Q = 1.00–1.09(–1.11), Qx = 1.05, globose to occasionally subglobose, distinctly punctuate/ echinulate, slightly thick-walled, yellowish brown to pale brown in KOH. BASIDIA 17–25 × 7–8.5 µm, clavate, mainly 2-spored. PLEUROCYSTIDIA absent. Lamella edge sterile, with crowded cheilocystidia. CHEILOCYSTIDIA 20–35 × 8–10 µm, mostly fusoid to utriform/lageniform, very infrequently ellipsoid to somewhat more or less sinuous, thin walled, hyaline. PILEIPELLIS a cutis with hyphae approx. 5 µm wide, radially oriented, walls moderately thick, hyaline, colorless. LAMELLA TRAMA regular, ≤ 5 µm wide. CLAMP CONNECTIONS numerous.

HABITAT: gregarious on rotten wood in a fragment of tropical rain forest.

MATERIAL EXAMINED: **BRAZIL. PERNAMBUCO**, Igarassu, Usina São José ("Mata de Piedade"), 25.viii.2005, *F. Wartchow s.n.* (URM 80088); Moreno, v.2008, RPPN Garnijó, *G.M. Mueller et al. 27* (URM 80089).

NOTE: The macroscopic features were noted from both collections; microscopic characters were taken almost entirely from URM 80088, except for observations of cheilocystidia in URM 80089.

REMARKS: This *C. flavus* collection is characterized by small, sulfur yellow basidiomes with slightly tomentose to smooth pilei, globose to occasionally subglobose basidiospores, clamped hyphae, exclusively 2-spored basidia, and absence of "antler-like" cheilocystidia (Capelari 2011). The last feature is consistent in the current collections as well as in material from São Paulo (Capelari 2011). All features fit with the species concepts given by Senn-Irlet & Immerzeel (2003) for this group.

In the protologue of *C. flavus*, Capelari (2011) tried to segregate this species from *C. sulphurinus* Imazeki & Toki, described from Japan, based on the two-spored basidia described for *C. sulphurinus,* although apparently no basidia

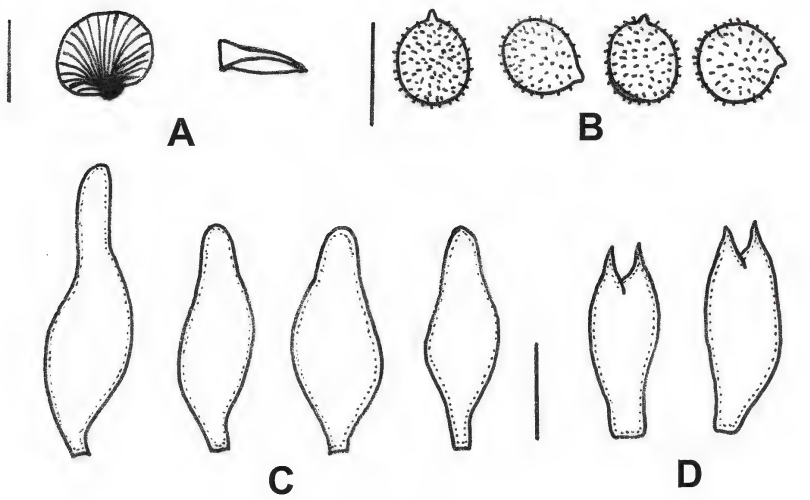

FIGURE 1. *Crepidotus flavus*.
A. Habit. B. Basidiospores. C. Cheilocystidia. D. Basidia.
Scale bars: A = 10 mm; B–D = 10 μm.

had been found in the original collection of *C. flavus*. According to Imazeki & Toki (1954), *C. sulphurinus* is a disjunct taxon that differs in the less globose basidiospores and somewhat clavate cheilocystidia. On the other hand, our materials agree with the protologue of the São Paulo material in the yellowish pileus and utriform/lageniform cheilocystidia (which Capelari interpreted as ventricose), although larger basidiospores (8.7 × 8.7 μm) were reported in the São Paulo material. Hongo (1961), who described *C. sulphurinus* specimens from Japan, referred to the cystidia as broadly clavate and ventricose, but the basidiospores as subglobose to globose (7.5–9 × 6.5–7.5 μm). Senn-Irlet & Immerzel (2003), however, reported utriform cystidia for other Japanese materials.

Another allied species is *C. citrinus* Petch from Sri Lanka. A type study by Pegler (1986) referred to clavate cylindric and often constricted cystidia, and apparently exclusively 4-spored basidia. Earlier, Singer (1973) reported *C. citrinus* as growing in montane subtropical forest in Argentina; he did mention the type from Sri Lanka but did not describe it. The Argentinean material and our *C. flavus* collection have a similar spore size (6.8–8.3 μm) but differ in the clavate to versiform cheilocystidia with frequent projections ("antler-like" in Senn-Irlet & Immerzeel 2003) and the 2–4-spored. Although Pegler (1986) did not report "antler-like" cystidia in the type and Pilát (1950)

had not referred to that feature, Senn-Irlet & Immerzeel (2003), who studied *C. citrinus* collections distant from the type locality (La Réunion and Puerto Rico), observed this feature. Special attention must be given to de Meijer (2008: 321), who reported rare antler-like cystidia in a Paraná collection of *C. citrinus* (he described them as having a "contorted apical appendage"). We follow the Senn-Irlet & Immerzeel (2003) species concept of this group and agree with Capelari (2011): we regard *C. flavus* as distinct from —but very similar to— *C. citrinus* and *C. sulphurinus* mainly by the cystidial shape. Morphological, phylogeographical, and molecular studies on a worldwide scale are necessary to elucidate whether or not all of these yellow species are different.

Crepidotus brunswickianus Speg. is another South American yellow species closely related to *C. flavus*. It was first described from Southern Argentinean forests by Spegazzini (1887) with a "pallide melleus" to "fulvo lutescens" pileus, concolorous lamellae, and basidiospores measuring 5–6 × 3–4 µm. It was later described from a montane zone in Venezuela as having cadmium yellow basidiomata, larger (7–8.5 × 6–7 µm) subglobose basidiospores, and vesicular to clavate cheilocystidia (Dennis 1961, 1970). Despite the close similarities in the descriptions, we believe that *C. brunswickianus* and *C. flavus* represent distinct species, although a revision is needed for the material identified in Dennis (1961).

Pereira (1990), Pegler (1997), Senn-Irlet & de Meijer (1998), de Meijer (2006, 2008) and Capelari (2007, 2011) reported several species of *Crepidotus*, and now we have the opportunity to record *C. flavus* for the first time in Northeast Brazil.

Entoloma aripoanum Dennis, Bull. Soc. Mycol. Fr. 69: 196. 1953. FIG. 2

PILEUS ≤ 17 mm, plane-convex, with a central depression, surface white, glabrous, smooth; margin smooth, entire, slightly involute; context thin, fleshy, white, unchanging. LAMELLAE adnate to short decurrent, white, membranous, moderately crowded, with lamellulae. STIPE 36 × 3 mm, central, cylindrical, white, glabrous, smooth, with rhizomorphs, mycelium present at base. ODOR strong, pleasant.

BASIDIOSPORES 10–11.5(–12.5) × 7–7.5 µm, x = 10.7 × 7.3 µm, Q = 1.30–1.50(–1.66), Qx = 1.45, heterodiametric-elliptical, with 6–8 depressed facets, pink and moderately thick-walled. BASIDIA 32–42 × 10–11 µm, clavate, 2- or 4-spored. PLEUROCYSTIDIA absent. CHEILOCYSTIDIA 47–72 × 9–17 µm, cylindrical and some clavate, hyaline, thin walled. PILEITRAMA of exclusively hyphae similar to ones of lamella trama; vascular hyphae occasional, 5 µm wide. PILEIPELLIS a cutis with erect hyphae 3.5–10 µm wide, hyaline. LAMELLA TRAMA regular with hyphae ranging to 3.5–8.5 µm wide, apparently with yellowish brown granular cytoplasmatic contents. CLAMP CONNECTIONS present.

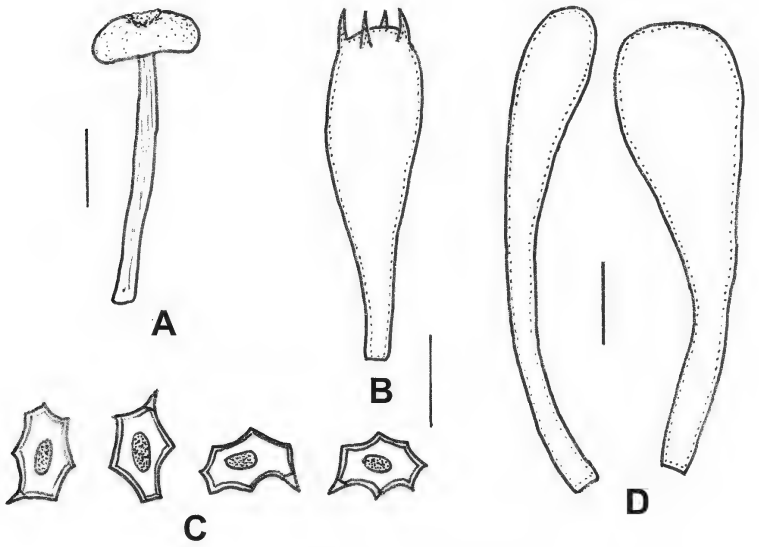

Figure 2. *Entoloma aripoanum*.
A. Basidiome. B. Basidium. C. Basidiospores. D. Cheilocystidia.
Scale bars: A = 10 mm; B–D = 10 µm.

Habitat: Solitary on rotten decayed wood in a tropical forest fragment.
Material examined: **BRAZIL. Pernambuco**, Igarassu, "Usina São José" ("Mata de Piedade"), 25.viii.2005, *F. Wartchow s.n.* (URM 80085).

Remarks: This is an interesting species recognized by the pure white basidiome with slightly depressed pileus centre, adnate to short decurrent lamellae, lignicolous habit, basidiospore size and shape, and clavate cheilocystidia (Baroni & Lodge 1998, as *Alboleptonia aripoana* (Dennis) Pegler). Cheilocystidia in our collection are slightly shorter than those in the Trinidad type as revised by Baroni & Lodge (1998), who reported cystidia 46–110 µm long, but the other features agree with the original description. Baroni & Lodge (1998) suggest that *A. aripoana* sensu Pegler (1983) represents a distinct species, based on the number of spore facets (8–10 in Pegler's Antillean specimen versus 6–8 in *E. aripoanum*). *Entoloma aripoanum* is known from Trinidad (Dennis 1953, Horak 1977), possibly also from Martinique and Dominica (Pegler 1983; but see Baroni & Lodge 1998), and now is reported for the first time from Brazil.

Entoloma tucuchense Dennis, Bull. Soc. Mycol. Fr. 69: 196. 1953. Fig.3

Pileus 35 mm, plane slightly convex, surface dark brown (M&P 8A12 "Autumn") radially striate-rimose showing the white context, disrupting in small granular squamules; context thin, fleshy, unchanging. Lamellae adnate,

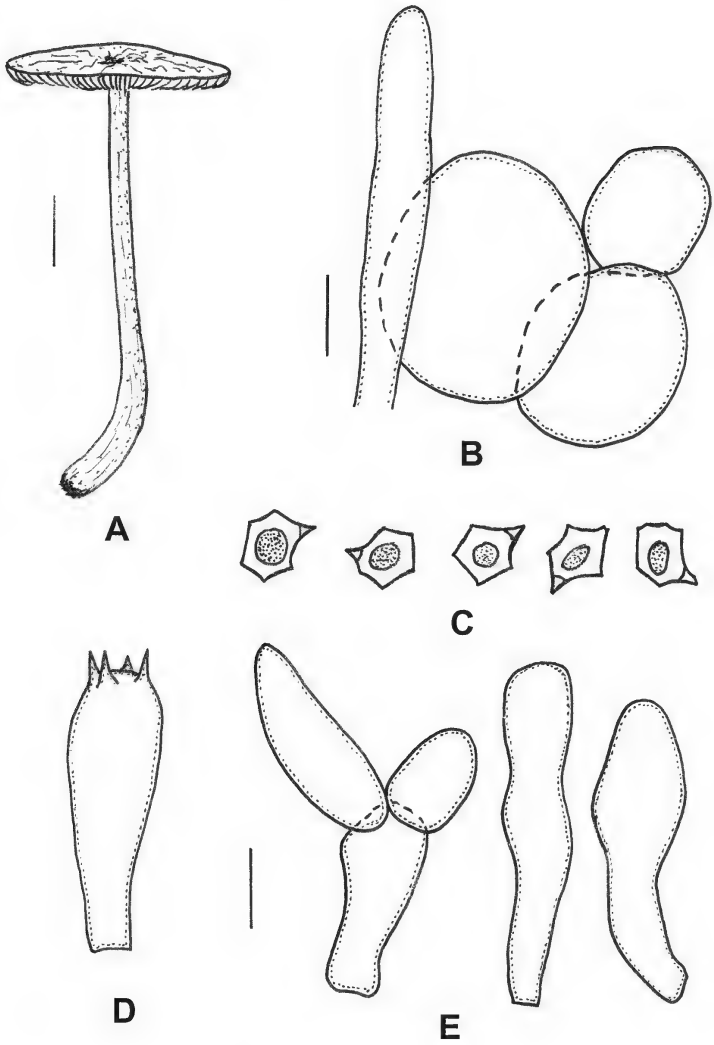

FIGURE 3. *Entoloma tucuchense*.
A. Habit. B. Pileipellis showing the inflated cells and tip on one of the erect cylindrical hyphae. C. Basidiospores. D. Basidium. E. Hyphoid terminal projections from lamella edge.
Scale bars: A = 10 mm; B–E = 10 μm.

pale cinnamon pink, membranous, sub distant, with lamellulae. STIPE 66 × 4 mm, central cylindrical, surface pale grey, smooth, white at base because of the basal mycelium.

Basidiospores 8–9.5(–10) × 6.2–7.5 µm, x = 8.7 × 7 µm, Q = (1.13–) 1.18–1.30(–1.35), Qx = 1.24, subisodiametric-angular, with 5–6 facets, with thin pink walls. Basidia 35–40 × 11–12 µm, clavate, 4-spored. Pleurocystidia absent. Cheilocystidia absent, although pronounced catenate hyphae 27–52 × 7–13 µm, frequently cylindrical, hyaline to light yellowish brown pigmented sometimes arising from the lamellar trama. Pileitrama hyphae filamentous or inflated, ≤ 11 µm diam.; 5-µm vascular elements occasionally present. Pileipellis an epithelium composed of subglobose to ovoid elements 18–37 × 15–26 µm intermixed with dark brown erect cylindrical hyphae 52–82 × 7–15 µm. Lamella trama regular, hyphae filamentous, ≤ 4 µm diam. Clamp connections absent.

Habitat: solitary on soil near an unidentified angiosperm in a fragment of tropical rain forest.

Material examined: **BRAZIL. Pernambuco**, Igarassu, Usina São José ("Mata de Piedade"), 22.vii.2005, *F. Wartchow 18/2005* (URM 80086).

Remarks: This species and *Calliderma fibulatum* Karstedt & Capelari, *C. rimosum* Karstedt & Capelari, *C. caeruleosplendens* Largent et al., *Entoloma foldatsii* (Dennis) E. Horak, and *E. pruinatocutis* E. Horak are all characterized by a granulose (rimose in this case) pileus and distinctly hymeniform pileipellis, a very infrequent feature in *Entoloma* sensu lato (Horak 1977, Aime et al. 2010, Karstedt & Capelari 2010). Except for the lack of cystidioid projections in the type specimen, other features (i.e. radially sulcate pileus, basidiospore size) our material matches the type as analyzed by Horak (1977), Pegler [1983, as *Inopilus tucuchensis* (Dennis) Pegler], and Karstedt & Capelari (2010).

The recently described *Calliderma rimosum,* the most macroscopically similar taxon, differs from *E. tucuchense* in a pileus that cracks more strongly with age, a revolute pileal margin, sinuate (not adnate) lamellae, and cylindric, clavate to ventricose cheilocystidia (Karstedt & Capelari 2010).

Entoloma tucuchense, previously known from Trinidad (Dennis 1953) and Amazonas State, Brazil (Horak 1982), is newly reported from Pernambuco State, northeast Brazil.

Recent studies place *E. tucuchense* and other taxa with hymeniform pileipelli in *Calliderma* (Romagn.) Largent (Aime et al. 2010, Karstedt & Capelari 2010). However, other recent molecular studies support *Calliderma* as an integral part of a monophyletic *Entoloma* (Co-David et al. 2009).

Lepiota erinana Dennis, Kew Bull. 7: 484. 1952. Fig. 4

Pileus 6 mm, convex, surface covered with appressed vinaceous brown (M&P 4K11 "Lacquer R") squamules, cracking to reveal a pale cream background, but remaining entire at centre, margin entire, not sulcate, nor striate; context very thin, submembranous. Lamellae free, membranous, white, crowded, with lamellulae. Stipe 9 × 1 mm, central, cylindrical, surface concolorous with the

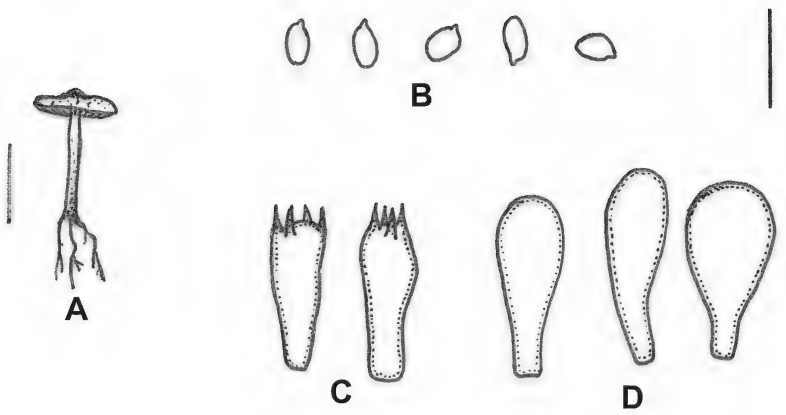

Figure 4. *Lepiota erinana*.
A. Basidiome. B. Basidiospores. C. Basidia. D. Cheilocystidia.
Scale bars: A = 5 mm; B–D = 10 µm.

pileus, with loose, floccose vinaceous brown squamules, rhizomorphs present. Annulus superior, ephemeral, and indistinct.

Basidiospores 4.5–5 × 2.5–3 µm, x = 4.6 × 2.7 µm, Q = (1.53–)1.62–1.78 (–1.88), Qx = 1.24, ellipsoid to elongate, thin walled, smooth, dextrinoid, hyaline. Basidia 15–17 × 5–6 µm, clavate, 2- or 4-spored. Pleurocystidia absent. Cheilocystidia 18–20 × 6–9 µm, inflate-clavate to clavate, thin walled, hyaline. Pileus covering a trichodermium comprising chains of cylindrical to subclavate hyphae with slightly thick walled light grayish terminal elements 37–47 × 5–6 µm. Lamella trama regular. Clamp connections present.

Habitat: solitary on humus in a fragment of tropical rain forest.

Material examined: **BRAZIL. Pernambuco**: Igarassu, Usina São José ("Mata de Piedade"), 22.vii.2005, *F. Wartchow 22/2005* (URM 78709).

Remarks: As far as we know, this small fragile species with a pileus ≤ 10 mm is known only from the Neotropics (Dennis 1952, Pegler 1983, de Meijer 2006, Rosa & Capelari 2009) and the very small size of our basidiome adds to what is known for *Lepiota erinana*. Known previously from Trinidad (Dennis 1952), Martinique and Venezuela (Pegler 1983), and Paraná (de Meijer 2006) and Minas Gerais (Rosa & Capelari 2009) states in Brazil, *L. erinana* is reported here for the first time from northeast Brazil.

Trogia cantharelloides (Mont.) Pat., Essai Taxon. Hymén.: 129. 1900.

Solitary on leaves and soil arising from a dense mycelium in litter in a tropical rain forest fragment.

Material examined: **BRAZIL. Pernambuco:** Igarassu, Usina São José ("Mata de Piedade"), 18.viii.2005, F. Wartchow (URM 78707).

Remarks: Singer (1965) previously reported *T. cantharelloides* was from Paraíba State, northeast Brazil. This is the first record from Pernambuco.

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Tremelloscypha gelatinosa (*Sebacinales*) from tropical deciduous *Gymnopodium* forests in southern Mexico

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Abstract — Populations of *Tremelloscypha gelatinosa* were found growing on the soil in *Gymnopodium floribundum* associations of the tropical deciduous forest in southern Mexico (State of Chiapas). Documentation of the different developmental coriaceous and spongy stages revealed a widely variable gross morphology that ranges from stipeless pulvinate forms to rudimentarily stipitate pseudoinfundibuliform forms that appear polyporoid, steroid, or gomphoid. The studied collections are described and their taxonomically distinctive macro- and micromorphological characters are illustrated. The possible mycorrhizal association of *T. gelatinosa* with *Gymnopodium floribundum* and traditional use of the basidiomes as a wild edible fungus by the local population are also discussed.

Key words — edible mushrooms, *Sebacinaceae*, taxonomy, tropical fungi

Introduction

During the last few years we have sampled ectomycorrhizal macromycetes within the *Gymnopodium floribundum* Rolfe (*Polygonaceae*) association, a type of tropical deciduous vegetation established in the Central Chiapas basin (State of Chiapas) in southern Mexico [Miranda 1952, as *G. antigonoides*; Reyes-García & Souza 1997]. Several species of *Amanita*, *Cantharellus*, *Russula* and other ectomycorrhizal genera have been found, all possibly associating with *G. floribundum*. *Lactarius chiapanensis* Montoya et al. was previously recorded from this ecosystem (Montoya et al. 1996) and recently several new fresh collections were gathered.

An interesting find in the *Gymnopodium* forest was numerous specimens of a fungus with coriaceous soft spongy rudimentarily stipitate sporophores. Mature specimens, which were somewhat trumpet-shaped, resembled a stout *Gomphus* species. After examination, we identified these specimens as

Tremelloscypha gelatinosa, a heterobasidiomycete related to members of order *Sebacinales* M. Weiß et al. (Weiß et al. 2004a). Molecular-based studies indicate that *T. gelatinosa* belongs to *Sebacinales* group A and is phylogenetically related to proven ectomycorrhizal species (Weiß & Oberwinkler 2001, Weiß et al. 2004a, Selosse et al. 2002). There is still no documentation about the mycorrhizas of *T. gelatinosa*, but its phylogenetic placement among recognized ectomycorrhizal sebacinoid species suggests that it could be a potential fungal symbiont of plant roots (Glen et al. 2002, Selosse et al. 2002, Urban et al. 2003, Weiß et al. 2004a,b). Considering the abundance of *T. gelatinosa* and its possible ectomycorrhizal status in the *Gymnopodium* forest that we visited, we collected mycorrhizas from soil samples, including some occurring directly under *T. gelatinosa* sporophores. Morphological examination revealed the constant presence of at least two different morphotypes, but as PCR amplification from extracted genomic DNA failed, it was not possible to determine whether the collected mycorrhizas involved *T. gelatinosa* or other fungi. New collections of root tips of *Gymnopodium floribundum–Tremelloscypha gelatinosa* will be studied in the future.

Tremelloscypha gelatinosa is rare not only in Mexico but also worldwide, and its morphological variation still deserves to be documented and illustrated. Therefore we present important information on the extent of morphological variation exhibited by fresh basidiomes collected at different developmental stages. Today the species concept of *T. gelatinosa* is based on the type specimen (a portion of a basidiome) and some single collections from Jamaica (type locality), Florida (USA), Yucatan and Quintana Roo (Mexico) (Burt 1915, Guzmán 2004, Roberts 2006, Wells 1961, Wells & Oberwinkler 1982). Here we describe our new collections in detail, providing photographs and illustrations of basidiomes and distinctive micromorphological characters and taxonomic observations.

Tremelloscypha gelatinosa is an economically valuable wild edible fungus in the Central Chiapas basin, particularly near Tuxtla Gutierrez, Chiapas. In this region *T. gelatinosa* is locally known as "nangañaña" and —with the so-called "moni" *Lactarius chiapanensis* (first reported by Miranda 1952 and formally described by Montoya et al.. 1996)— is commonly collected by the local population for personal consumption and/or for sale. *Gymnopodium floribundum* is a dominant or co-dominant species in the deciduous tropical forest established at SE of Tuxtla Gutierrez (Miranda 1952, Reyes-García & Souza 1997); the *G. floribundum* forest, which (although now fragmented) covers several hectares, is collectively called "nangañal" ("aguanal" or "aguanacatonal" in Miranda 1952). More will be published on traditional use of *T. gelatinosa* in the future.

Materials & methods

Observations were made from fresh basidiomes collected in a forest dominated by *Gymnopodium floribundum*, a tropical deciduous component of vegetation in Suchiapa, municipality of Suchiapa, SE of Tuxtla Gutierrez, Chiapas. Color codes in descriptions refer to Kornerup & Wanscher (1967; e.g. 10C2) and Munsell (1994; e.g. 5YR 4/1). For microscopic observations, hand sections of dried samples were mounted in 3% KOH and stained with both 1% Congo red aqueous solution and phloxine. Line drawings were made with the aid of a drawing tube. Measurements were made in KOH; 35–45 spores were measured per collection; *Xr* = range of means of length × width of n collections; *Qr* = range of means of basidiospore length/width ratios in n collections. Herbarium acronyms follow Holmgren et al. (1990).

Identification of collection VB4212 was confirmed by extraction and amplification of the ITS rDNA region according to Montoya et al. (2010). The obtained sequence was edited with Sequencher Ver. 4.1 (Gene Codes, Ann Arbor, Michigan) and deposited in GenBank database (http://www.ncbi.nlm.nih.gov/) under accession number *T. gelatinosa* (VB 4212) JQ012947. The ITS rDNA sequence from VB4212 was compared with *T. gelatinosa* AF490394 (GenBank) using the BLAST sequence similarity search tool (Altschul et al. 1997), revealing a 99% maximum similarity.

Taxonomy

Tremelloscypha gelatinosa (Murrill) Oberw. & K. Wells,
Mycologia 74: 325, 1982. PLATES 1–4

≡ *Eichleriella gelatinosa* Murrill, Ann. Missouri Bot. Gard. 2: 748, 1915.

BASIDIOMES (25–)40–165 × 30–110 mm, pulvinate or irregularly subglobose in young stages, becoming more or less trumpet-shaped in profile and then pseudoinfundibuliform, rudimentarily stipitate, soft and spongy when wet but coriaceous and even fibrous in dried condition. PILEUS (25–)40–165 mm wide, subglobose, gradually plano-convex, plane and finally plane-depressed (not deeply depressed or perforated), then pseudoinfundibuliform, irregularly circular, frequently becoming flabellate or spathulate, often confluent forming an irregular mass of spathulate or flabelliform pilei, with somewhat crenate or more or less lobed margin, this latter thin or broad, slightly elevated, obtuse; surface dry, zonate, at first velvety, with age appearing fibrillose, villose or strigose, at times somewhat floccose-scaly (mainly in old specimens); whitish or grayish when young, at times whitish with pale gray-blue or pinkish areas, becoming brown-yellow, brownish (5D5–7), brown-reddish or brown-orange (6D4–6), rarely with greenish shades, with darker concentric zones (6E6–7, 6F5–6), in dried material pale brown to brown, with spongy aspect, dry; margin whitish becoming brownish or paler, in some stages showing pale vinaceous tinges. HYMENOPHORE decurrent, smooth, plane to irregularly rugulose or with short bulges, then at times appearing faintly verruculose, continuous, rarely cracked, cartilaginous in aspect, tough when dried, whitish or with yellow to

Plate 1. *Tremelloscypha gelatinosa*. (a: Bandala 4210; b: Bandala 4212). Scale bar = 20 mm.

Plate 2. *Tremelloscypha gelatinosa*. (a: Bandala 4361; b. Bandala 4363). Scale bar = 20 mm

gray tints (5YR 4/1), dark or light grayish-vinaceous (9D3, 9E4, 10C2, 11D2), gray with pink tinges (5YR 2/5–6) or grayish-brown (5D2-3, 5E3, 6C2, 6D2–3), occasionally pale orange-brown (5YR 3–4/3–4) or pale orange (7B5), whitish, pale vinaceous or pale vinaceous-brown towards or on the margin, often with obscure concentric zones. Stipe as an attenuated end of the hymenophore attached to the substratum, at times with age becoming poorly defined, around 15–25 × 15–30 mm, more or less cylindrical to attenuate, stout, velutinous, somewhat hirsute to glabrous at base, same color with pileus or hymenophore, whitish at base, compact. Context pale to more or less grayish-white, often with pale vinaceous or pink tinges, showing concentric growth lines, dry, fibrous, unchanging on exposure, 6–21 mm thick in pileus. Odor strong, almost farinaceous (recalling some stipitate hydnoids), taste mild.

Basidiospores 8–13 × (5.5–)6–9 µm, (*Xr* = 9.1–11.5 × 6.8–7.4 µm; *Qr* = 1.28–1.58), ellipsoid to broadly ellipsoid, some subglobose, at times adaxially weakly depressed, apiculus conspicuous, more or less truncate, thin-walled, smooth, hyaline, guttulate, germination not observed. Basidia 14–18 × 9–12 µm, ovate to obovate, at times clavate or subglobose, forming 2–4 basidial segments, clampless, guttulate; epibasidia cylindric or faintly tapered apically, up to 45 × 2–4 µm; fertile hyphae faintly tortuous, usually branching, forming basidia by proliferating near subbasidial hyphal segment. Dikaryophyses simple to shortly branched, 2–4 µm diam., guttulate, abundant, not forming a distinct layer above the basidia; the basal portion of the hymenial elements consisting of cylindrical, septate and compactly arranged hyphae; the general aspect of the hymenial stratum is of a gelatinous tissue, with its elements very compactly arranged and with refractive contents, more or less differentiated into several growth layers, each consisting of prostrate and ascending elements. Context consisting of cylindric, yellowish-brown or pale brown hyphae, 3–5(–9) µm wide, simple or bifurcate, shortly or more or less spacing segmented, thick-walled, loosely interwoven or in some areas with many hyphae arranged in tight fascicles forming a compact tissue; a narrow, prostrate layer composed of indistinct, agglutinated hyphae giving rise to the hymenial elements. Clamp connections absent.

Ecology— Scattered or gregarious (occasionally solitary) in soil, commonly among fallen dead leaves and twigs, the basidiomes often enclosing small, dead twigs or thin stalks of live plants, in tropical deciduous *Gymnopodium floribundum* forest.

Material studied — **MEXICO. Chiapas**: Suchiapa, Mpio. de Suchiapa, 4.XI.2003, *Bandala 3838*; 5.VII.2005, *Montoya 4353*; 5.IX.2006, Bandala *4210*, *4212* (GenBank JQ012947); 14.VI.2008, *Bandala 4361*, *4363*; 15.VI.2008, *Bandala 4366*, *4370*, *4374* (all at XAL). **JAMAICA**. Troy and Tyre, Cockpit Country, *W.A. Murrill & W. Harris* (Fungi of Jamaica 1087) (Holotype, NY).

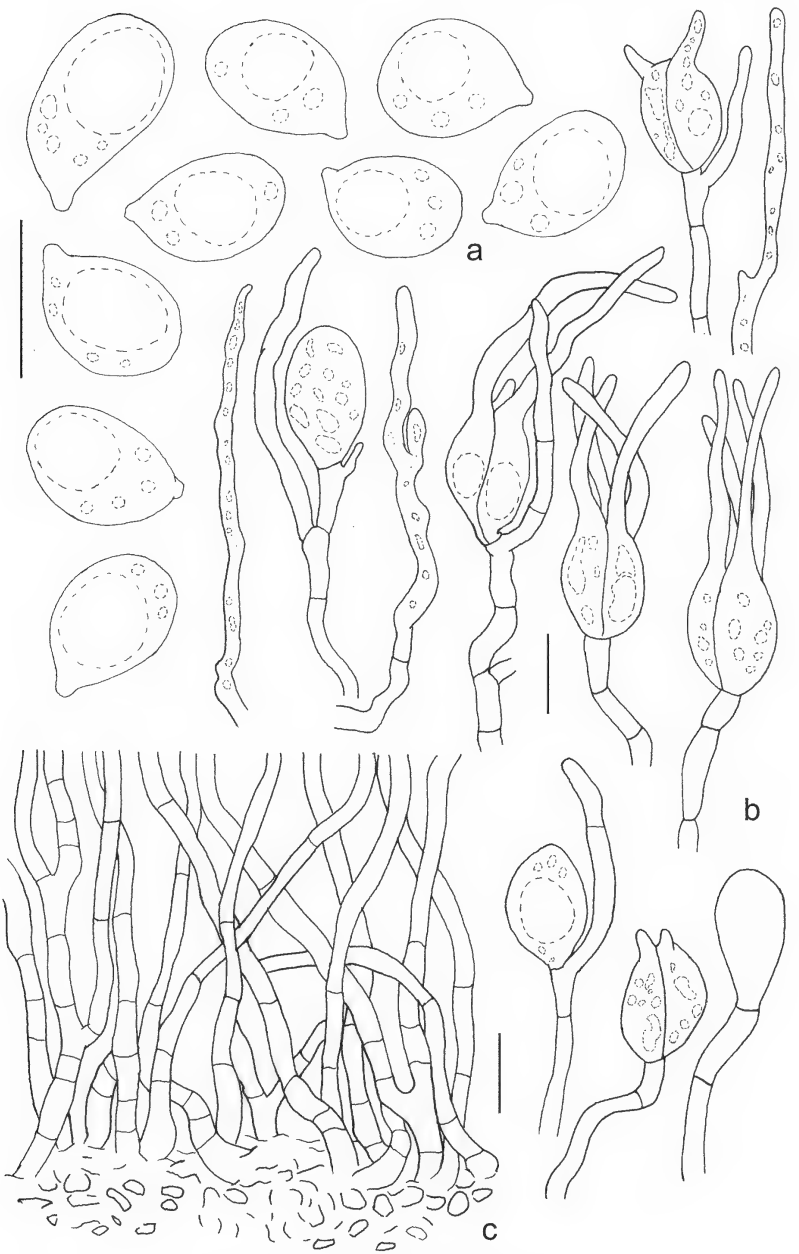

PLATE 3. *Tremelloscypha gelatinosa*.
a. Basidiospores. b. Basidia and dikaryophyses. c. Section of basal portion of hymenium. (Bandala 4210). Scale bar = 10 μm

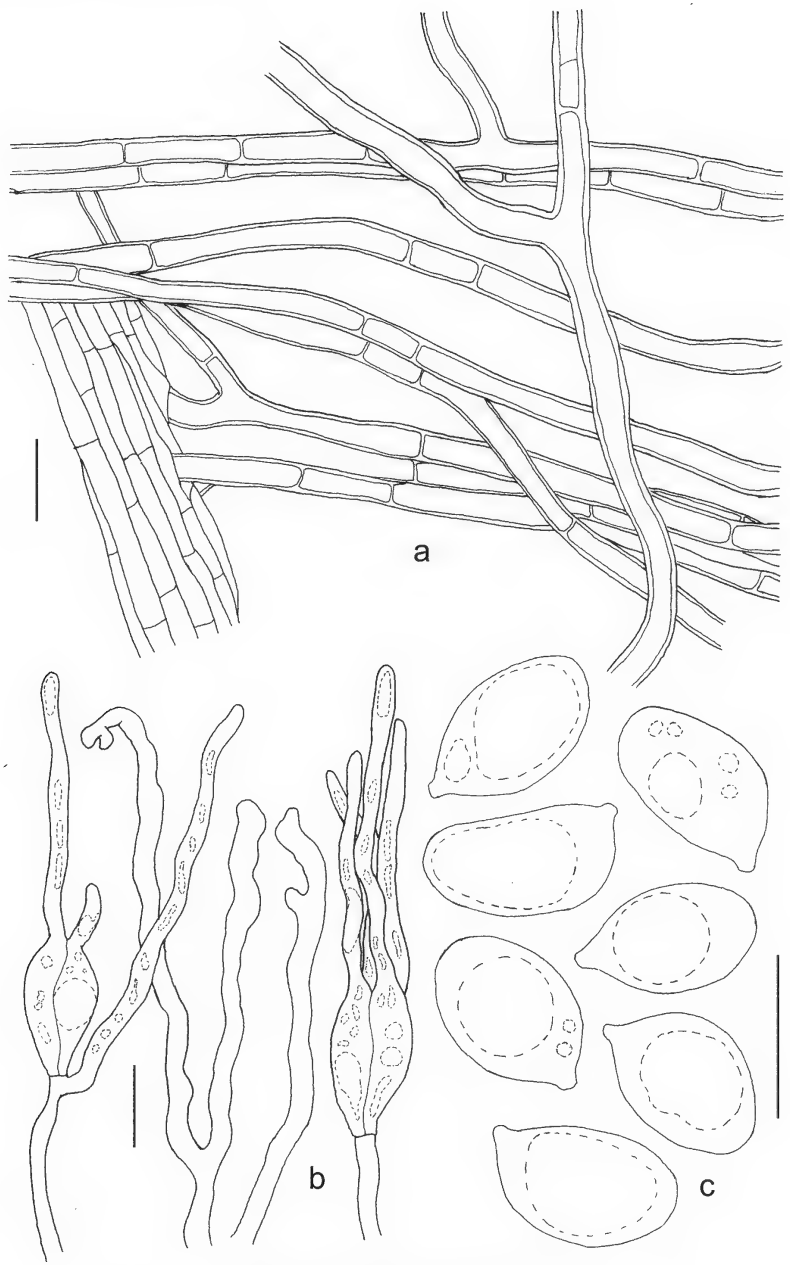

Plate 4. *Tremelloscypha gelatinosa*.
a. Context hyphae. b. Basidia and dikaryophyses. c. Basidiospores.
(a: Bandala 4210; b–c: Bandala 4274). Scale bar = 10 μm.

Discussion

Recognition of *Tremelloscypha gelatinosa* as a non-resupinate fungus (i.e. different from members of *Eichleriella* Bres.; cf. Wells & Raitviir 1980) was inferred not from the type but from a specimen from Florida gathered by K. Lampe & A.L. Welden in 1957 (Wells 1961). The holotype (from Jamaica) in fact consists of only a pressed 35 × 18 mm portion apparently separated from the rest of the basidiome and superficially bearing a resemblance to an effuse-reflexed fructification as indicated both by the diagnosis (Burt 1915) and the field label accompanying the type noting "a specimen of *Stereum* or *Cladoderris*." Using data from the previously cited Florida collection and microscopic examination of the holotype, Wells & Oberwinkler (1982) recorded *T. gelatinosa* as growing on decayed wood and described the basidiomes as 35–75 × 20–55 mm, 1–6 mm thick, flabellate, infundibuliform or pseudoinfundibuliform in shape, and with a poorly defined stipe.

Our fresh collections covered many different developmental stages, thus providing more information on the variability of the gross morphology. The fructifications were irregularly subglobose, pulvinate, lacking a stipe or rudimentarily stipitate and pseudoinfundibuliform, more or less trumpet-shaped and sometimes flabellate or spathulate, not with umbilicate, depressed or deeply depressed pileus, in some stages the sporophores then superficially resembled polyporoid, stipitate-steroid or gomphoid forms.

Sporophores of both *T. australiensis* D.A. Reid, the type species of the genus (Reid 1979), and our *T. gelatinosa* collections grew on soil, not wood. *Tremelloscypha australiensis* differs morphologically from *T. gelatinosa* in its smaller (7–20 × 8–12 mm) distinctly infundibuliform stipitate basidiomes that are yellow when fresh and yellowish- or ochraceous-brown when dry and a pileus surface with an almost waxy-cartilaginous appearance (Reid 1979). Spore characters are particularly important: *T. australiensis* basidiospores are subcylindrical, narrowly elliptical to suballantoid (Reid 1982: 10–13.2 × 4.5–5 µm; Wells & Oberwinkler 1982: 10.5–13 × 5–5.5 µm), while *T. gelatinosa* basidiospores are ellipsoid to broadly ellipsoid (Burt 1915: 8–10 × 6 µm; Wells 1961: 7.5–11 × 5.5–8 µm; Wells & Oberwinkler 1982: 9–13.5(–14.5) × 5.5–8(–9.5) µm; Guzmán 2004: (7–)9–13 × 6–8 µm; this study: 8–13 × (5.5–) 6–9 µm).

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for providing information on local use of *T. gelatinosa* as a wild edible fungus. We appreciate the collaboration of Biols. P. del Moral and D. Ramos in the laboratory and in the field, and MSc E. Garay by assistance during the molecular analysis (all at Instituto de Ecología, Xalapa). This work was partially supported by project Monitoreo de aves en líneas de transmision fase III. Chicouasen, Chiapas, Juile, Veracruz (directed by R. Villegas).

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New records of polypores from southern Florida

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Abstract — Fifteen new records of polypore species are reported from the southern Florida, USA, with short comments to their key features, ecology, and distribution. The combination *Fomitiporia apiahyna* is proposed.

Key words — *Basidiomycota*, *Hymenochaetaceae, Polyporaceae,* taxonomy, invasive species

Introduction

The southernmost part of Florida south of Tamiami Trail (U.S. 41, which bisects Miami) lies in the tropical hardwood forest region of the state (Young & Giese 2003). This relatively small area contributes substantially to poroid xylophilous fungi diversity in the USA, and about 15% of USA polypore species published in a compendium by Gilbertson & Ryvarden (1986, 1987) probably are found only here. Although Ryvarden (2004) and Decock et al. (2007) published new data on southern Florida *Ganodermataceae* and *Hymenochaetaceae,* to our knowledge no new records have been added for the *Polyporaceae* since 1987.

Most of the territory belongs to Everglades National Park and is covered with regularly flooded wetland or with extensive mangrove stands along the coast. The rather uniform mangrove polypore flora from Brazil treated by Sotão et al. (2002) and Trierveiler-Pereira et al. (2009) is not the primary focus of our study.

Much higher polypore diversity is found in tropical hardwood forest areas with red-barked gumbo-limbo (*Bursera simaruba* (L.) Sarg. (*Burseraceae*)) the typical tree species. This forest-type, however, is confined to small tear-shaped

islands of "hardwood hammocks" that occur on raised peaty platforms above surrounding wetlands. As most are inaccessible by foot, the casual visitor is restricted to only a handful of such localities in the Everglades and several Miami city parks. Another distinctive Everglades locality comprises the remnant pine rockland fragments dominated by *Pinus elliottii* Engelm. (*Pinaceae*).

Several small state parks were also established in the Florida Keys archipelago. Their polypore flora is not as rich because many temperate tree species, such as southern live oak (*Quercus virginiana* Mill. (*Fagaceae*)) and sugarberry (*Celtis laevigata* Willd. (*Ulmaceae*)), together with their associated polypores reach their southern limits on the mainland. Also, Florida Keys hardwood hammocks are drier because of ocean breezes and low rainfall (Whitney et al. 2004). On the other hand, there are several tropical trees that occur only in the Keys and some polypore species seem to be growing only on them.

After exploring all these small localities several times, we have discovered some tropical polypores not yet recorded from the USA. We describe these below and also propose one new combination. Critical Florida collections were compared with our finds of the same or similar species from northern Florida, US Virgin Islands, Mexico, Belize, and Guatemala. Some species could be determined only after ITS rRNA region sequencing. Surprisingly, sequencing confirmed some of the most common polypores as new records.

Materials & methods

During December 2003, April 2009, August 2010, and December 2010, polypore specimens were photographed in situ, collected, dried, and examined microscopically mounted in Melzer´s reagent or 10% KOH. All specimens were deposited in private herbarium of the first author, with duplicates of some specimens maintained in the Prague Museum Herbarium (PRM).

DNA was isolated from selected polypore samples and the ITS rRNA region was sequenced to confirm the determination. 0.25 g of the context tissue from dried specimens was disintegrated 60 s with a steel ball in mixer mill MM301 RETSCH at room temperature. DNA was isolated using CTAB/NaCl extraction buffer (Murray & Thompson 1980), followed by repeated extraction with chloroform and isopropanol precipitation. Crude DNA was dissolved in 100 ml of sterile water and further purified using Wizard Clean Up kit PROMEGA. Resulting DNA solution (50 µl) was diluted ten times and 1 µl was used as template for amplification with ITS5 and ITS4 primers (White et al. 1990) in 25 ml reaction mixture using 55 °C annealing temperature. Amplified DNA was sequenced in the Genomics laboratory of Biology Centre, Academy of Sciences of the Czech Republic, České Budějovice, on ABI 3730xl DNA analyzer, using BigDye Terminator 3.1 kit.

Results & discussion

Noteworthy collections are listed alphabetically by family, genus, and species. Descriptions are included for some species, with additional comments

on distribution, ecology, and diagnostic or critical characters. All species are new records to Florida.

Hymenochaetaceae Donk

Fomitiporia apiahyna (Speg.) Vlasák & Kout, **comb. nov.** PHOTO 1

MYCOBANK MB 519982

≡ *Fomes apiahynus* Speg., Bol. Acad. Nac. Ci. 11: 438, 1889.

Basidiomes perennial, sessile, triquetrous, ungulate, 3 × 2 × 1 cm; upper surface glabrous, sulcate, zonate, dark brown to black, margin acute, pore surface grayish-brown, the pores circular, 7–9 per mm, with thick, entire dissepiments; tubes 2 mm long each year, context yellowish brown, woody hard, up to 0.2 cm thick. Hyphal system dimitic, contextual skeletal hyphae brown in KOH, thick-walled, rarely branched, 2.5–5 µm in diam.; generative hyphae hyaline, thin-walled, 1.5–3 µm in diam., with simple septa. Basidia broadly ellipsoid, 7–10 × 4.5–6 µm, simple septate at the base, with 4 sterigmata. Basidiospores globose, hyaline, thick-walled, dextrinoid in Melzer´s reagent, 5–6 × 4.5–5 µm.

SPECIMENS EXAMINED – **USA. FLORIDA**: COLLIER COUNTY, Fakahatchee Strand Preserve, East Main Tram, 23. IV. 2009, sabal palm, leg. J. Vlasák Jr., det. J. Vlasák (JV1008/113), (JV1008/114); MONROE COUNTY, Florida Keys, Long Key State Park, 27. VIII. 2010, shrub, leg. & det. J. Vlasák (JV1008/46); SARASOTA COUNTY, Myakka River State Park, 24. IV. 2009, sabal palm, leg. & det. J. Vlasák (JV1008/147A).

DISTRIBUTION & ECOLOGY – from northern Argentina to Costa Rica (Ryvarden 2004). In southern Florida not rare, most often on living sabal palm (*Sabal palmetto* (Walter) Lodd. ex Schult. (*Arecaceae*)) but also on other substrates.

PHOTO 1: *Fomitiporia apiahyna* JV1008/113 on living sabal palm.

Comments – This species is remarkable for its very small thumbnail-sized applanate (more often ungulate) pilei. Its microscopic features are reminiscent of *Fomitiporia robusta* (P. Karst.) Fiasson & Niemelä: globose thick-walled strongly dextrinoid spores that are, however, distinctly smaller in *Fo. apiahyna* and an absence of setae. As molecular analysis clusters the species with *Fo. robusta/punctata* (Góes-Neto et al. 2002), we transfer this species to *Fomitiporia* Murrill as defined by Fiasson & Niemelä (1984).

Fomitiporia maxonii Murrill

Description – Ryvarden (2004:188).

Specimens examined – **USA. Florida:** Dade County, Miami, Fairchild Botanical Garden, 24. XII. 2003, leg. J. Vlasák Jr., det. J. Vlasák (JV0312/24.16-J, PRM915956); **US Virgin Islands:** St. John, 3. IX. 2004, leg. J. Vlasák Jr., det. J. Vlasák (JV0409/19-J, PRM915955).

Distribution & ecology – Costa Rica, Belize, Galapagos (Ryvarden 2004). Probably quite rare in the USA: we collected it only once in Florida (in Fairchild Botanical Garden) and also on US Virgin Islands. On dead hardwoods.

Comments – The species resembles *Fo. punctata* (P. Karst.) Murrill but has somewhat larger pores and smaller spores. It can be positively determined only after DNA sequencing. Sequences of our collections deposited in the GenBank (GU136210, GU136211) correspond perfectly to other published *Fo. maxonii* sequences (Decock et al. 2007).

Inonotus micantissimus (Rick) Rajchenb. Photo 2

Description – Ryvarden (2004: 136).

Photo 2: *Inonotus micantissimus* JV1008/80 on living *Ocotea coriacea*.

Specimens Examined – **USA. Florida:** Dade County, Miami, Matheson Hammock Nature Area, 30. VIII. 2010, *Ocotea coriacea*, leg. J. Vlasák, det. L. Ryvarden (JV1008/80, ITS rRNA JF692194); **US Virgin Islands:** St. John, 3. IX. 2004, leg. K. Vlasáková., det. J. Vlasák (JV0409/3-K, PRM902083). **JAMAICA**. Ocho Rios, 12. VI. 2008, leg. J. Vlasák Jr., det. J. Vlasák (JV0806/18-J).

Distribution & ecology – Argentine, Brazil, and Dominican Republic (Ryvarden 2004). We have collected this species before on US Virgin Islands and Jamaica; the ITS rRNA region sequences of these collections are nearly identical. All collections are from living hardwoods.

Comments – *Inonotus micantissimus* may be recognized by its resupinate or somewhat bulbous, very dark brown basidiomata, abundant setal hyphae as well as hymenial setae and large, globose, hyaline basidiospores, 10–13 μm in diam.

Fuscoporia callimorpha (Lév.) Groposo, C.L. Leite & Góes-Neto Photos 3–5

Descriptions – Ryvarden & Johansen (1980:145); Loguercio-Leite & Wright (1995).

Photo 3: *Fuscoporia callimorpha* JV0904/87, in situ.

Specimens examined – **USA. Florida:** Broward County, Davie, Tree Tops Park, 26.IV.2009 (JV0904/174, 178); 28.XII.2003 (JV0312/28.5-J); Everglades Nat. Park, Long Pine Key Campground, 27.VIII.2010 (JV1008/43); 29.VIII.2010 (JV1008/63); Everglades Nat. Park, Royal Palm, 19.IV.2009 (JV0904/33); 22.IV.2009 (JV0904/87, ITS rRNA JF692190&191, JV0904/88); 20.XII.2003 (JV0312/20.1-J, 20.5-J, ITS rRNA JF692192); 28.XII.2003 (JV0312/28.5-J); Dade County, Miami, Matheson Hammock Nature Area, 26.VIII.2010, leg. & det. J. Vlasák (JV1008/10); 19.IV.2009 (JV0904/13–16); 24.XII.2003 (JV0312/24.2-J,24.8-J); Highlands County, Highlands Hammock

Photo 4: *Fuscoporia callimorpha* JV0904/155, in collection.

St. Park, 25.IV.2009 (JV0904/155); Saint John County, St. Augustine, Washington Oaks St. Park, 20.XII.2002 (JV0212/7-J); Sarasota County, Myakka River St. Park, 24.IV.2009 (JV0904/143, 144); **US Virgin Islands**, St. John, 4.IX.2004, leg. J. Vlasák Jr., det. J. Vlasák, (JV0409/14-J, ITS rRNA JF692193, 15-J). **BELIZE**. Cockscomb Basin, 30.X.2006, leg. J. Kout, det. J. Vlasák (JV0610/13P-K). **GUATEMALA**. Tikal, 4.XI.2006, leg. J. Kout, det. J. Vlasák (JV0611/1P-K, K3C-K). **MEXICO**. **Veracruz**: Los Tuxtlas, 12.X.2006, leg. J. Kout, det. J. Vlasák (JV0610/S-K); **Chiapas**: Palenque, Campground, 19.X.2006, leg. J. Kout, det. J. Vlasák (JV0610/6P-K). **VENEZUELA**. Cueva el Guacharo, 22.II.2004, leg. J. Kout, det. J. Vlasák (JV0402/41, 42).

Distribution & ecology – Africa (Ryvarden & Johansen 1980), Central America (Lowe 1957), Brazil (Loguercio-Leite & Wright 1995). On dead hardwoods.

Comments – This annual, sometimes reviving, corky hard species is by far the most common pileate *Phellinus* in southern Florida. Accordingly, it is also a very variable species forming usually flat applanate pilei but sometimes also triquetrous or effused-reflexed basidiomes or fully effused (on the log underside) with detached sharp margin. No cuticle or black line develops under the pileus tomentum, which is quite persistent, usually structured in sharp zones (agglutinated in some), exuding a thin waxy layer that is somewhat shiny-glossy and silvery. This important field trait is, however, not obvious in old pilei with hardened surfaces. The pore surface is much more constant: fulvous

Photo 5: *Fuscoporia callimorpha* JV0312/20.1 and JV0312/20.5, in collection.

with a purplish tint and pores that are very small (7–9 per mm), very regular, round, and with thick dissepiments. Hymenial setae are short (≤ 25 μm), not much contrasting, abundant in some specimens but very rare and difficult to find in others. In the hymenium are thin-walled, ventricose cystidioles (necks ≤ 10 × 1.5-2 μm) that are not mentioned in descriptions but well pictured in Loguercio-Leite & Wright (1995). Basidiospores (oblong ellipsoid, hyaline, thin-walled, 3.5–4.2 × 2.2–2.9 μm) are always collapsed and difficult to find in winter and early spring collections.

This species is evidently a taxonomical problem. The ITS rRNA region sequence is identical with the GenBank sequence AY558649 from culture CBS 182.34. In 1934 Overholts isolated the culture in the USA as *Phellinus torulosus* (Pers.) Bourdot & Galzin. Tomšovský & Jankovský (2007) showed, however, that this sequence differs from the European *Fuscoporia (Phellinus) torulosa* (Pers.) T. Wagner & M. Fisch. Moreover, *Fu. torulosa* develops much thicker basidiomes that are very persistent, even on dead wood for more than 25 years. *Fu. torulosa* probably does not occur in the USA, for there are no reliable records (see also Rizzo et al. 2003) and we were not able to find it despite numerous excursions in Pennsylvania and Virginia where it should have optimal conditions for growth.

Phellinus roseocinereus (Murrill) D.A. Reid, described from North America and widespread in Central America (Lowe 1957), corresponds very well to this common Florida polypore. However, we agree with Ryvarden & Johansen (1980) and Loguercio-Leite & Wright (1995), who consider *P. roseocinereus*

identical to *Fu. callimorpha*, described originally from Madagascar. Corner (1991) was unable to detect any difference between *Phellinus senex* (Nees & Mont.) Imazeki from Asia and *P. callimorphus,* except for slightly narrower spores of the latter. The sequence of *Fuscoporia senex* (Nees & Mont.) Ghob.-Nejh. in GenBank AY558647 (isolate CBS 442.76 of B.K. Bakshi from India), which is really much more similar to our sequences than *Fu. torulosa,* still clusters separately (with 99% bootstrap support, not shown). Judging from the literature and our examinations of two *Fu. senex* specimens from China, we think that *Fu. senex* has only an indistinctly zonate pileus surface, lacks glossy zones, and produces broader spores (>3.5 μm; compared to always <3 μm in *Fu. callimorpha*). Also, older *Fu. senex* spores are sometimes slightly thick-walled and yellowish, never true in *Fu. callimorpha.*

Phellinus neocallimorphus Gibertoni & Ryvarden, known only from the type locality in Brazil, differs from *Fu. callimorpha* only by the absence of setae. Nevertheless, we also could not find any setae in some of our specimens (e.g. 0312/20.5) although in other respects, including rDNA ITS region sequence, the specimens were typical. More detailed study of *P. neocallimorphus* is needed, evidently.

Phellinus gilvus (Schwein.) Pat. is another quite similar species found in southern Florida, although not as often as elsewhere in the USA. The similar basidiomes are, however, glabrous, without tomentum, azonate, and softer. Although also hyaline and thin-walled, *Ph. gilvus* spores are ovoid and 3–3.5 μm broad.

We have sequenced nine collections of *Fu. callimorpha* from southern Florida, US Virgin Islands, and Mexico with markedly different pileus forms, and, except for a few mutations, all sequences are the same. We infer that there is no other similar species in the region.

Phellinus calcitratus (Berk. & M.A. Curtis) Ryvarden PHOTO 6

DESCRIPTION – Ryvarden (2004: 162).

SPECIMENS EXAMINED – **USA. FLORIDA**: BROWARD COUNTY, Davie, Tree Tops Park, 26.IV.2009 (JV0904/167); DADE COUNTY, Miami, Matheson Hammock Nature Area, 30.VIII.2010, leg. & det. J. Vlasák (JV1008/83, ITS rRNA JF894114, JF894115); 26.VIII.2010 (JV1008/11); 19.IV.2009 (JV0904/12).

DISTRIBUTION & ECOLOGY – South America, West Indies (Ryvarden 2004). Locally abundant in the Matheson Hammock Nature Area but rare elsewhere. Always on rather thick, old, decorticated logs, probably oaks.

COMMENTS – This is a characteristic species with a broadly attached, applanate pileus with a sharp margin and thick crustulose black zone under hard tomentum. The very thin-walled tubes are unique for *Phellinus* species in the region. Spores are thick-walled, globose, yellow to brown. The sequence shows no homology with GenBank sequences.

Photo 6: *Phellinus calcitratus* JV1008/83.

Phellinus caribaeo-quercicola Decock & S. Herrera Photo 7

Description – Decock et al. (2006).

Specimens examined – **USA. Florida:** Broward County, Davie, Tree Tops Park, 26.IV.2009, leg. & det. J. Vlasák (JV0904/177, ITS rRNA GU594159); Everglades Nat.

Photo 7: *Phellinus caribaeo-quercicola* JV0904/28-J.
(The photo was taken one year after breaking off the original basidiome.)

Park, Royal Palm, 19.IV.2009, leg. J. Vlasák Jr., det. J. Vlasák (JV0904/28-J, PRM915960, ITS rRNA GU594158); 20.XII.2003 (JV0312/20.7-J).

Distribution & ecology – Described relatively recently from western Cuba as growing exclusively on *Quercus cubana* A. Rich. (Decock et al. 2006). Nevertheless, it seems not to be rare around Miami on dead stems and branches of *Q. virginiana*.

Comments – *Phellinus caribaeo-quercicola* produces large perennial bulbous or semipileate basidiomes, usually with several layers of rather long tubes. The rare to abundant thick-walled setae with hooked tips and subglobose thick-walled light yellowish spores are characteristic. The ITS rRNA of our collections corresponds perfectly to the published sequence (Decock et al. 2006).

Phellinus nilgheriensis (Mont.) G. Cunn. Photo 8

Description – Ryvarden & Johansen (1980:187).

Specimens examined – **USA. Florida**: Dade County, Hwy 41, Kirby Storter Boardwalk, 28.VIII.2010, leg. & det. J. Vlasák (JV1008/49); (JV1008/50); 23.IV.2009 (JV0904/112, PRM915957).

Distribution & ecology – Southeast Asia, Africa, Cuba (Ryvarden & Johansen 1980), French Antilles, and Guiana (David & Rajchenberg 1985), Brazil (Groposo et al. 2007); always on hardwoods. Not previously published from the USA. However, around the Kirby Storter boardwalk at a Hwy 41 wayside, the fungus grows on several living *Taxodium distichum* (L.) Rich. trees in the slough, approximately at the height of summer-time water level.

Comments – The applanate pilei, not as hard as in similar *Phellinus* species, are typically more or less glabrous with a narrow rounded margin and rather bright

Photo 8: *Phellinus nilgheriensis* JV1008/50 on living *Taxodium distichum*.

brown context. Lack of setae and subglobose thick-walled, yellow to rusty brown spores (in Melzer's) may remind of *Fomitiporia* when viewed in Melzer's reagent. It seems to be annual but reviving species— new tubes overgrow dead the previous year's tubes with the tube layers separated by a context tissue layer. The ITS rRNA of our collections corresponds perfectly to published sequence AY558633.

Polyporaceae Fr. ex Corda

Coriolopsis hostmannii (Berk.) Ryvarden — Photo 9

Description – Ryvarden (2007).

Specimens examined – (all on living buttonwood mangrove) **USA. Florida:** Collier County, Hwy 41, Collier-Seminole State Park, 24.XII.2003, leg. J. Vlasák, det. L. Ryvarden (JV1008/56, 57); 23.IV.2009, leg. & det. J. Vlasák (JV0904/125); 25.XII. 2003, leg. J. Vlasák Jr., det. J. Vlasák (JV0312/25.7-J); Monroe County, Florida Keys, Long Key State Park, 27.VIII.2010, leg. J. Vlasák, det. L. Ryvarden (JV1008/42).

Distribution & ecology – Pantropical. This species is probably confined to living buttonwood (*Conocarpus erectus* L. (*Combretaceae*)). Its ecology was extensively treated in several papers about buttonwood polypore populations (Bergeman et al. 2009, Parrent et al. 2004) using the name "*Datronia caperata* (Berk.) Ryvarden from buttonwood", even though the authors were aware that their fungus is not conspecific with typical *D. caperata*. Our ITS rRNA sequence

Photo 9: *Coriolopsis hostmannii* JV1008/42 on living buttonwood.

corresponds very well with their published sequences and differ from an authentic *D. caperata* sequence.

Comments – This is a very typical and relatively common species. Nonetheless, it appears in the mycological literature quite rarely under its proper name. It seems to be often misinterpreted, for example as *Datronia caperata*, a South American species with similar spores and pores but with a dark brown context, tough trametoid consistency, and coarsely hirsute pileus. *Coriolopsis hostmannii* looks more like a pileate *Inonotus* species because of its relatively soft context and bright brownish colors in all parts of basidiome. The pileus surface is glabrous to shiny-glossy, the pores are very small and regular, and the pore surface is characteristically uneven.

Coriolopsis polyzona (Pers.) Ryvarden

Description – Ryvarden & Johansen (1980: 291).

Specimens examined – **USA. Florida:** Dade County, Florida City, irrigation canal dam, 29.VIII.2010, *Ficus* sp., leg. J. Vlasák Jr., det. J. Vlasák (JV1008/64-J). **BELIZE.** Cockscomb Basin, 30. X. 2006, hardwood, leg. J. Kout, det. J. Vlasák (JV0610/A8-K). **JAMAICA.** Ocho Rios, 12.VI.2008, hardwood, leg. J. Vlasák Jr., det. J. Vlasák (JV0806/12-J). **MEXICO. Veracruz:** Montepio, 14.X.2006, hardwood, leg. J. Kout, det. J. Vlasák (JV0610/A16-K); Los Tuxtlas, 12.X.2006, hardwood, leg. J. Kout, det. J. Vlasák (JV0610/A14A-K).

Distribution & ecology – Pantropical, on dead hardwoods (Ryvarden & Johansen 1980:291). We collected this species many times in Mexico, Belize, and Jamaica but only once in Florida, in a typical man-influenced locality.

Comments – Common in South America and resembling the boreal *Trametes hirsuta* (Wulfen) Lloyd but with even more pronounced and more hirsute zones on pileus surface and larger, less regular pores that are often a bit elongated in various directions. The broader spores of *C. polyzona* are narrowly elliptic but not cylindric.

Navisporus floccosus (Bres.) Ryvarden Photo 10

Description – Torres-Torres et al. (2007).

Specimen examined – **USA. Florida:** Dade County, Miami, Old Cutler road alley, hardwood stump, 26.VIII.2010, leg. & det. J. Vlasák (JV1008/30, ITS rRNA JF692195).

Distribution & ecology – Africa, Southeast Asia (Ryvarden & Johansen 1980), Cuba, Mexico (Torres-Torres et al. 2007), Costa Rica (Mata et al. 2007).

Comments – Remarkable, very large (≤ 50 cm wide × 10 cm thick) pilei reminding large *Ganoderma* P. Karst. distinguish this species. The glabrous pileus surface has a patchy black cuticle or thin azonate crust. The thick light brown context has a relatively soft consistency and is strikingly lightweight when dry. Because of large naviculate spores, this species is traditionally classified close to *Navisporus sulcatus* (Lloyd) Ryvarden, also a South Florida

Photo 10: *Navisporus floccosus* JV1008/30.

species, but very different, with small, effused-reflexed pilei and relatively large pores. Decock (2007) has commented critically on the taxonomical position of *N. floccosus*.

Skeletocutis diluta (Rajchenb.) A. David & Rajchenb. Photo 11

Description – David & Rajchenberg (1992).

Specimens examined – **USA. Florida**: Dade County, Everglades Nat. Park, Long Pine Key, Campground, 29.VIII.2010, *Pinus elliottii* log, leg. & det. J. Vlasák (JV1008/61, ITS rRNA JF692198); Long Pine Key, Pinelands Trail, 20.IV.2009, *Pinus elliottii* log, leg. & det. J. Vlasák (JV0904/44); (JV0904/45). **BELIZE**. Cockscomb basin, 30.X.2006, hardwood, leg J. Kout, det. J. Vlasák (JV0610/16-K, ITS rRNA JF692197); 29.X.2006, hardwood, leg J. Kout, det. J. Vlasák (JV0610/G4-K).

Distribution & ecology – Argentina, Africa (David & Rajchenberg 1992), Panama (Núñez & Ryvarden 1999). Mostly on hardwoods but the type was collected in Argentina on *Pinus taeda* L. (Rajchenberg 1983). In Florida it occurs on logs of slough pine (*Pinus elliottii*) in seasonally flooded parts of the Everglades Long Pine Key region. Probably not rare as we noted several quite disintegrated basidiomes in dry wintertime. During the next summer the whole area was a half meter under water and we could collect only one well developed specimen just on the edge of a water basin.

Comments – Dextrinoid to weakly amyloid skeletals that dissolve in KOH and the tiny spores make this species unmistakable. The species was originally described as "resupinate or effused-reflexed." Although our collections from Belize on hardwoods are always pileate, our Florida specimens (collected on pine) are strictly resupinate. Also, from the microphotography, the spores

Photo 11: *Skeletocutis diluta* JV1008/61 on *Pinus elliottii* log.

seem to be only 2.5 × 0.4 µm, smaller than published (3.1–3.5 × 0.5–0.8 µm). Nevertheless, the ITS rRNA sequences of Belize and Florida samples are nearly identical. Though originally described as a variety of *S. nivea* (Rajchenberg 1983), the ITS rRNA sequence shows no significant homology with *S. nivea* (Jungh.) Jean Keller but low homology with *S. chrysella* Niemelä (91%) and *S. kuehneri* A. David (90%).

Trametes lactinea (Berk.) Sacc.

This species is treated separately in Vlasák & Kout (2011).

Trametes ochroflava Cooke Photo 12

= *Daedalea microsticta* Cooke

Description – Fidalgo & Fidalgo (1967: 848).

Specimen examined – **USA. Florida**: Dade County, Miami, Fairchild Botanical Garden, *Albizia* stump, 24. XII. 2003, leg. J. Vlasák Jr., det. J. Vlasák (JV0312/24.17-J, PRM915968).

Distribution & ecology – South and Central America, Mexico, West Indies. Dead deciduous trees (Ryvarden & Aime, unpublished).

Comments – *Trametes ochroflava* is characterized by large, flat, tough, whitish pilei with labyrinthine pores and brownish context and trama. Pores often appear also on pileus surface. The species was long known as *Daedalea microsticta* and only recently has the older and more appropriate name been

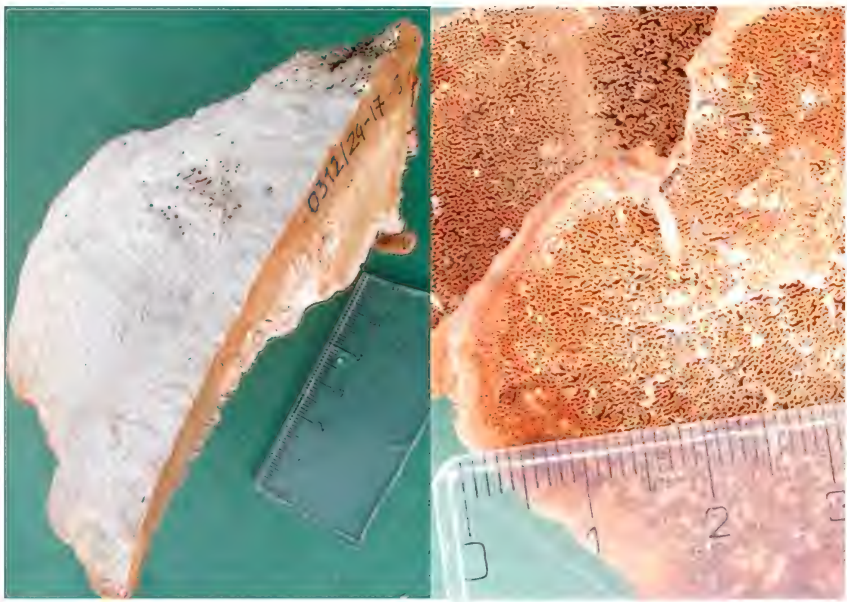

Photo 12: *Trametes ochroflava* JV0312/24.17 in collection.

applied (Ryvarden & Aime, unpublished). The type of rot is unknown but the sequence shows similarity to *Trametes* Fr., not *Daedalea* Pers. The sequence of our specimen is identical with the published sequence FJ403209.

Ceriporiopsis cystidiata C.L. Leite, G.V.C. Gonç. & Ryvarden

Description – Loguercio-Leite et al. 2001.

Inconspicuous, white resupinate polypore with 2–3 mm long tubes and very thin subiculum, margin and touched places somewhat yellowish when dry.

Specimen examined – **USA. Florida**: Dade County, Miami, Matheson Hammock Nature Area, 26.VIII.2010, hardwood, leg. & det. J. Vlasák (JV1008/26).

Distribution & ecology – up to now known only from the type locality: Brazil, dead hardwood.

Comments –The abundant hymenial cystidia and cylindrical but rather thick straight spores are diagnostic.

Wrightoporia bracei (Murrill) I. Lindblad & Ryvarden Photo 13

= *Amylosporus bracei* (Murrill) A. David & Rajchenb.

Description – Murrill 1921, Rajchenberg 1983.

Strikingly pinkish-violet resupinate polypore, widely effused on the substrate, with tiny pores (5–7 per mm). Spores amyloid, ovoid, 3.5–4 × 2.5–3 finely

Photo 13: *Wrightoporia bracei* JV1008/77 in situ.

echinulate, hyphal system dimitic, skeletal hyphae weakly dextrinoid, generative hyphae with simple septa.

Specimen examined – **USA. Florida**: Monroe County, Florida Keys, Key Largo, John Pennekamp Coral Reef State Park, 29.VIII.2010, hardwood, leg. & det. J. Vlasák, (JV1008/77, ITS rRNA JF692199).

Distribution & ecology – Argentina (Rajchenberg 1983), French Antilles (David & Rajchenberg 1985), Bahamas, Puerto Rico (Murrill 1921). Tropical hardwood forest.

Comments— Contrary to original description, this specimen is strongly rhizomorphic, perhaps due to the growth on an underside of a log and its violet colors are unchanged after drying. The species was transferred to *Amylosporus* Ryv. by David & Rajchenb. (1985), but the rRNA ITS sequence shows only low homology with *Wrightoporia lenta* (Overh. & J. Lowe) Pouzar and *W. luteola* B.K. Cui & Y.C. Dai (GenBank) and none at all with *Amylosporus campbellii* (Berk.) Ryvarden (our sequences JF692200, JF692201).

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Paraphysoderma sedebokerense, gen. et sp. nov., an aplanosporic relative of *Physoderma* (*Blastocladiomycota*)

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Abstract — A monocentric blastocladialean fungus was identified as a parasite of the green alga *Haematococcus pluvialis* in aquaculture and isolated into pure culture. Phylogenetic analysis suggests the fungus is a sister taxon to the obligately biotrophic plant parasitic genus *Physoderma*. The parasite, which has not been observed to reproduce by zoospores, instead forms amoeboid aplanospores. We formally describe the new genus and species *Paraphysoderma sedebokerense* to accommodate this unusual member of the *Blastocladiomycota*.

Key words — algae, *Blastocladiales*, cultures, nomenclature

Introduction

A fungal parasite of the green alga *Haematococcus pluvialis* has recently been characterized (as "*Haematococcus* parasite" in Hoffman et al. 2008) and provisionally referred to as "*Paraphysoderma sedebokerensis*" (Gutman et al. 2009; Porter et al. 2011). The parasite has the appearance of a typical chytridiomycete zoosporic fungus with an epibiotic globular sporangium subtended by an intracellular rhizoidal system. It was first observed during aquaculture of *H. pluvialis*, an important producer of the carotenoid astaxanthin used in cosmetics and pharmaceuticals (Del Campo et al. 2007). Neither pure nor dual cultures of the alga and fungus produced a zoosporic stage; instead sporangia released amoeboid aplanospores that were infective to *H. pluvialis* (Hoffman et al. 2008) but not other green algae (Gutman et al. 2009). In a molecular phylogeny based on the 18S ribosomal RNA gene the novel parasite was closely related but clearly basal to the plant parasitic zoosporic fungus *Physoderma* (*Blastocladiomycota*) (Hoffman et al. 2008). Its phylogenetic

position, the absence of a zoospore stage, and its occurrence in an algal host suggest that the parasite should be placed in a separate genus.

A detailed description of the infection cycle and growth characteristics of the parasite has been published (Hoffman et al. 2008). The goal of this paper is to provide a formal description of the parasite.

Taxonomy

Paraphysoderma Boussiba, Zarka & T.Y. James, **gen. nov.**

MycoBank MB 561750

Sporangium monocentricum, eucarpicum, epibioticum. Aplanosporae paucae usque ad plurimae per sporangium, sine flagellis, congregentes antequam liberati, per incisuram in sporangio exeuntes, 3–6 µm diametro pseudopodiis filosis, in superficie cellulae nutricis repentes antequam incystati et endogene evolventes. Systema rhizoidale e puncto unico in sporangio evolvens, intra hospitem bulbosum. Sporangium dormiens epibioiticum, fuscatius, pariete crassiore quam zoosporangium, germinans ut aplanosporas liberarent. In alga viridi parasitica.

Type species — *Paraphysoderma sedebokerense* Boussiba, Zarka & T. James

Etymology — The new genus is closely related to, but distinct morphologically and in host preference, from the genus *Physoderma*.

Sporangium monocentric, eucarpic, epibiotic. Aplanospores few to dozens per sporangium, lacking flagella, swarming before being released, exiting through tear in sporangium, 3–6 µm diameter with filose pseudopodia, crawling on surface of host cell before encysting and developing endogenously. Rhizoidal system developing from single point on the sporangium, forming an apophysis inside host. Resting sporangium epibiotic, darker and with thicker wall than zoosporangium, germinating to release aplanospores. Parasitic on green algae.

Paraphysoderma sedebokerense Boussiba, Zarka & T.Y. James, **sp. nov.**

MycoBank MB 561751

Descriptio ad genus. In Haematococcus pluvialis *parasitica.*

Holotype — Hoffman et al. 2008, Mycological Research 112: Fig. 5D.

Etymology — Referring to Sede Boker, Israel, the locality where the species was originally isolated.

Description as for genus. Sporangium size 20–35 µm in dual culture on host (Hoffman et al. 2008); aplanospores with numerous refractive globules that coalesce upon encystment; parasitic on non-motile *Haematococcus pluvialis* Flot. (*Haematococcaceae*, *Chlorophyceae*).

Discussion

Paraphysoderma is differentiated from *Physoderma* by the absence of a motile spore, the presence of an epibiotic resting sporangium, culturability,

and host range. However, it has a general appearance of a typical monocentric, eucarpic chytrid or blastocladialean fungus, and is similar to *Physoderma* in having an epibiotic sporangium and a limited rhizoid emanating from a single axis on the sporangium. Its phylogenetic position (Hoffman et al. 2008) implies that the genus or species has recently lost a flagellated spore stage. Despite the absence of a motile spore, the aplanospores of the parasite are nonetheless able to cause severe epidemics in aquaculture.

Our current observations do not exclude the possibility that additional study of *Paraphysoderma* will ultimately reveal a zoospore stage. When sexuality is observed in members of phylum *Blastocladiomycota*, an alternation of diploid and haploid generations is observed (James et al. 2006). Thus it is possible that a thallus or stage of different ploidy on an alternative host may exist, although infection studies suggest it is not likely to be another green alga (Gutman et al. 2009). However, the observation that the aplanospores from both resting and thin-walled sporangia are morphologically similar suggests the absence of a separate gametophyte stage. If the fungus conforms to the life cycles expected of other blastoclads, then it would be considered to have a 'Brachyallomyces' (short) life cycle, which may or may not include meiosis during resting sporangial germination (Emerson 1941). The basal placement of *Paraphysoderma* to *Physoderma* is consistent with the derivation of the land plant parasitism from an aquatic one between a chytrid-like fungus and an alga. The culturability of *Paraphysoderma* and not *Physoderma* is consistent with the evolution of an increasingly obligate parasitic relationship of *Physoderma* to its host. Indeed, *Physoderma* and *Paraphysoderma* are unique among *Blastocladiomycota* in its parasitism of the plant kingdom, although whether the use of plant hosts is a derived or ancestral trait is unclear, as *Physodermataceae* occupy the most basal branch in the *Blastocladiomycota* (Porter et al. 2011). The distinct differences in culturability between the two genera suggests that *Paraphysoderma* might serve as a model species for the clade in research requiring large numbers of cultured cells, such as genomics and biochemistry.

The unique nature of the amoeboid swarming aplanospores suggests that neither genus nor species has been observed before. *Chytridiomycota* species that have been found to reproduce solely by amoeboid aplanospores include *Amoebochytrium rhizidioides* Zopf (host: *Chaetophora elegans* (*Chlorophyceae*)), *Sporophlyctis rostrata* Serbinow (host *Draparnaldia* spp. (*Chlorophyceae*)), and *Sporophlyctidium africanum* Sparrow (host *Protoderma* sp. (*Ulvophyceae*)), all of which differ in several ways (including rhizoidal development) from *Paraphysoderma*. An aplanosporic mode of reproduction is unusual among aquatic fungi, and the factors that favor the loss of a flagellated spore in an aquatic environment are unclear. The pattern that many aplanosporic chytrids parasitize green algae suggests that the need for a motile spore may be minimal

in certain well-mixed environments, while the ability to creep over the host to find an ideal location for rhizoidal penetration may the trait most influenced by natural selection.

The number of accepted species of *Physoderma* is ~50 (Kirk et al. 2008). The number of species and distribution of *Paraphysoderma* are currently unknown but will probably be much greater than currently appreciated given the rapid expansion of chytrid diversity seen in both culture studies and environmental DNA surveys (Letcher et al. 2008; Freeman et al. 2009).

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Microcyclospora rumicis, a new species on *Rumex crispus* from Iran

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Abstract — A new species of *Microcyclospora*, associated with leaf spots on *Rumex crispus*, is fully described and illustrated.

Key words — hyphomycetes, *Pseudocercospora*, weed, cercosporoid fungi

Introduction

During the study of alternative weed hosts for *Cercospora beticola* Sacc., a causal agent of cercospora leaf spot of sugar beet in Northern Iran, a species of the genus *Microcyclospora* J. Frank et al. was isolated from *Rumex crispus* showing leaf spot symptoms in the Talesh region (Guilan province). *Microcyclospora*, with *M. pomicola* J. Frank et al. designated as type species, was recently segregated from *Pseudocercospora* Speg. s. str. based on phylogenetic and morphological differences (Frank et al. 2010). Currently the three species known in *Microcyclospora* are associated with sooty blotch and flyspeck (SBFS) blemishes on the surfaces of pomaceous fruits, specifically apples (Colby 1920; Frank et al. 2010). Here we describe a new *Microcyclospora* species from leaf spots on *Rumex crispus* that differs morphologically from the three other known species of the genus.

Materials & methods

Conditions of isolation, culture, and observation

Leaf samples of *Rumex crispus* showing leaf spot were collected from Rainforest Mountains in the Talesh region, Guilan province, in northern Iran near the Caspian Sea. Single conidial isolates were established directly from symptomatic curly dock leaves according to Bakhshi et al. (2011). A mass of conidia scraped from the lesion using a sterile inoculation needle under a stereomicroscope was floated in 10 ml sterile distilled water and spread on 2% malt extract agar (MEA; Fluka, Germany). After plates

were incubated in a slanted position overnight, germinated conidia were transferred to new MEA plates, and cultures were incubated in the dark at 25 °C. After 30 days of incubation, samples from the colony were mounted on glass slides in clear lactic acid for microscopic examination. Thirty measurements were made for each microscopic structure, and 95% confidence intervals were derived for the measurements with extreme values given in parentheses. Line drawings were prepared using a BX41 light microscope (Olympus, Japan) equipped with a drawing tube; photos were captured using a Leica camera system. Colony colors on MEA and oatmeal agar (OA; Gams et al. 2007) [surface and reverse] were determined after one month at 25 °C in the dark. Nomenclature and descriptions were deposited in MycoBank. The holotype voucher and an ex-type culture are conserved in CCTU, the culture collection housed at the Plant Protection Department, Agriculture Faculty, University of Tabriz, Iran.

Taxonomy

Microcyclospora rumicis Arzanlou & Bakhshi, **sp. nov.** PLATE 1–2

MYCOBANK MB 563723

Microcyclospora rumicis *ab aliis speciebus generis chlamydosporis numerosis, majoribus, ad 20 µm diam distinguenda.*

TYPE: Iran, Guilan, Talesh, on leaves of *Rumex crispus* L. (*Polygonaceae*), Sep. 2010, M. Bakhshi (**Holotype**, CCTU-H-1; **ex-type culture**: CCTU 1 = CBS 131546).

ETYMOLOGY: Named after the host genus, *Rumex*.

Culture characteristics — On MEA slow growing, reaching 3 mm diam after 7 d, and up to 10 mm after 2 weeks at 25 °C, raised, unevenly folded, with moderate, smoke-gray aerial mycelium; surface irregular, with smooth, lobate margins, iron-grey; iron-grey in reverse. Colonies on OA reaching up to 2 mm diam after 7 d, and 6 mm after 2 weeks at 25 °C, flat, submerged, with sparse aerial mycelium and smooth margin. Microcyclic conidiation commonly observed on all media in culture.

In vitro on MEA — Mycelium consisting of branched, smooth, septate, pale brown, 2–4 µm wide hyphae developing numerous chlamydospores, intercalary and terminal, medium brown, 6–20 µm diam. Conidiophores reduced to conidiogenous cells, integrated, lateral on hyphae, mono- to polyblastic, subdenticulate, 2.5–4 µm wide, 8–13 µm tall, hyaline, smooth. Conidia scolecosporous, cylindrical, straight to variously curved, guttulate, apex obtuse, base truncate, hyaline, (1.5–)2.5(–4) × (15–)37–54(–100) µm, 1–10-septate; hila neither thickened nor darkened; microcyclic conidiation commonly observed; older conidia developing intercalary chlamydospores that are pale brown, ≤ 9 µm diam.

Discussion

The cercosporoids are amongst the major groups of fungi and generally cause leaf spot diseases on almost all families of flowering plants throughout

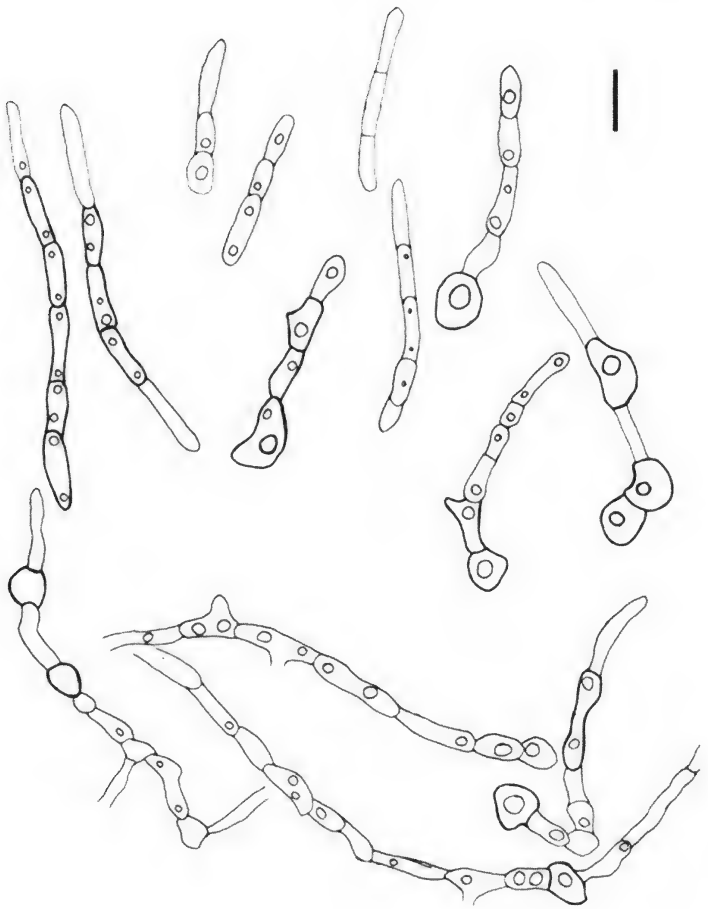

Plate 1. *Microcyclospora rumicis.*
Conidia, conidiogenous cells and hyphae from MEA colony. Bar = 10μm.

the world. Among these, *Pseudocercospora* is the second largest cercosporoid genus, with more than 300 published names (Kirk et al. 2008). Recently it was shown that *Pseudocercospora* included taxa that vary considerably in their conidiomatal morphology, ranging from solitary conidiogenous loci, synnemata, sporodochia to fascicles. Moreover, conidia in some taxa are transversely euseptate but with some oblique and longitudinal septa or containing a mixture of eu- and distoseptation. Conidial hila and scars vary from unnoticeable to slightly thickened along the rim (Stewart et al. 1999). Even though the conidia

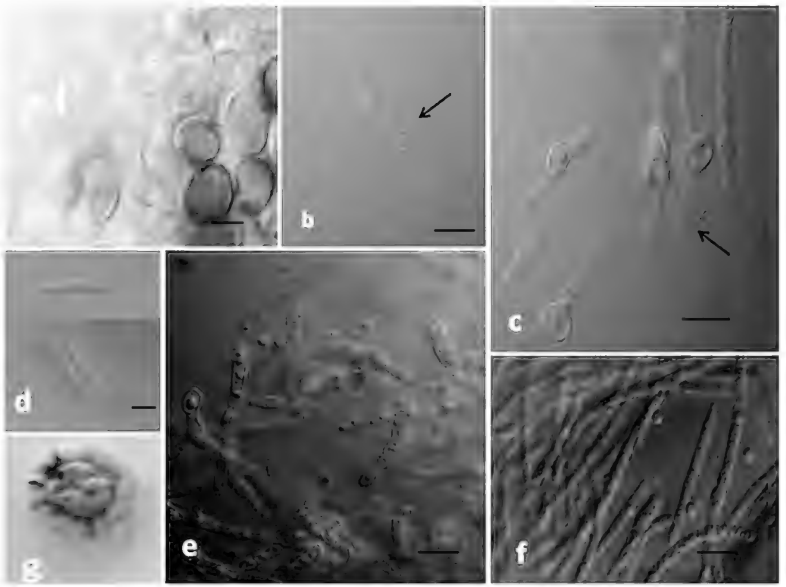

Plate 2. *Microcyclospora rumicis*. a. Chlamydospore in hyphae; b–c. Conidiogenous loci (arrowed) and conidia with chlamydospore; d. Young conidia; e–f. Hyphae. g. 30-day old colony on MEA. Scale bars = 10 µm.

are commonly solitary, they may in some cases also occur in unbranched chains (Braun 1995). Frank et al. (2010) established *Microcyclospora* based on morphological and molecular data. Morphologically, *Microcyclospora* can be distinguished from *Pseudocercospora* by the arrangement of the conidiophores that are never fasciculate but reduced to solitary conidiogenous loci on hyphae and conidia that aggregate in mucoid masses, strongly tending toward microcyclic conidiation. Only three other species described in the genus —*M. malicola* J. Frank et al., *M. pomicola,* and *M. tardicrescens* J. Frank et al.— associated with sooty blotch and flyspeck (SBFS) blemishes on surfaces of pomaceous fruits (Frank et al. 2010). Here, we introduce a new species based on morphological characteristics. *Microcyclospor*a *rumicis,* like *M. tardicrescens,* forms intercalary chlamydospores (lacking in *M. malicola* and *M. pomicola*) and grows more slowly in culture. Chlamydospores of *M. rumicis* are longer (≤ 20 µm) than those of *M. tardicrescens* (≤ 5 µm long).

Little is known about sexual stages of the cercosporoid fungi including *Microcyclospora* species. Cercosporoids have been traditionally treated as anamorphs of the ascomycete *Mycosphaerella* Johanson (e.g., Braun & Mel'nik 1997, Kim & Shin 1998, Crous & Braun 2003). However, most cercosporoids

are considered exclusively asexual, and *Mycosphaerella* teleomorphs have been confirmed for only a few species.

Phylogenies derived from multiple sequence analyses place *Microcyclospora* with other cercosporoid fungi in the *Mycosphaerella* clade (*Mycosphaerellaceae*, *Capnodiales*, *Dothideomycetidae*; Frank et al. 2010). *Mycosphaerella,* one of the largest genera in the *Ascomycota,* comprises several thousand species (Crous et al. 2001, 2009a,b; Aptroot 2006). Contrary to the assumption that *Mycosphaerella* as then defined was monophyletic, Crous and co-workers recently revealed that the genus is polyphyletic and split the former *Mycosphaerellaceae* into several families, of which the *Mycosphaerellaceae*, *Teratosphaeriaceae* and *Schizothyriaceae* have plant-pathological importance (Crous et al. 2007, 2009a). Among the vast number of anamorphs in these lineages, up to 30 anamorph genera have been linked to *Mycosphaerella* (Crous et al. 2007, 2009a; Arzanlou et al. 2007). Recent phylogenetic analysis based on multiple sequence data sets, however, indicate that these interpretations are not quite correct (Crous et al. 2009b) and that the *Mycosphaerellaceae* encompass instead numerous genera with morphologically conserved *Mycosphaerella*-like teleomorphs but quite distinct anamorphs (Crous et al. 2007, 2009b).

Little is currently known about ecology, host ranges, and sexual stages of the cercosporoid fungi including *Microcyclospora* species, and more sampling on different substrates is needed to address these aspects.

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Mycena guldeniana – a new alpine species from Norway

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Abstract — *Mycena guldeniana* (section *Polyadelphia*) is proposed for an agaric found in alpine areas of south Norway that is here described, illustrated and compared with other species of the section. The new species grows on fallen, decaying leaves under *Salix* spp. or occasionally on small twigs in the same habitat and is characterized by its small size, greyish brown pileus, 4-spored basidia, smooth cheilocystidia, prominent, wide terminal cells of the pileipellis hyphae, and clamp connections. Phylogenetic analyses of nucLSU support the taxon within *Mycena* and genetically distinct from the morphologically similar *M. terena*.

Key words — taxonomy, alpine mycology, basidiomycete phylogenetics

Introduction

During a stay at 'Finse Research Centre' in south Norway, August 2005, the first author collected and studied several *Mycena* species. The research centre is situated at approximately 60°30′N on the mountain plateau Hardangervidda 1200 m above sea level. The area lies entirely within the alpine zone and is devoid of trees but in parts is covered with *Salix* shrubs. The research led to the proposal of two new *Mycena* species (Aronsen & Gulden 2007). Two collections were also made of a small *Mycena* that initially was misinterpreted as *M. terena* Aronsen & Maas Geest., a species found on fallen, decaying leaves of *Salix caprea* in the coastal area of South Norway (Aronsen & Maas Geesteranus 1992).

In 2008 the 'Finse research centre' was visited again, and the main investigation was done on the south-facing slope at Nordnut at approximately 1400 m. The slope is formed by basic, phyllitic rocks intermixed with *Salix* shrubs and some calcicolous vegetation (Gulden & Jenssen 1982). On small, partially buried twigs on the ground under the *Salix* shrubs a tiny *Mycena* species was collected. Further investigation showed that not only was it identical with the taxon found in 2005, it clearly differed from *M. terena* and did not match any other known species of *Mycena*.

The same year this taxon was also collected at the base of the mountain Gaustatoppen at approximately 59°51′N at approximately 1150 m. Gaustatoppen (1883 m) is the peak of a 7 km long mountain spine consisting of quarzite, and in the lower slopes there are areas with dense *Salix* shrubs. In these *Salix* shrubs the unknown taxon was found in great numbers together with *M. exilis* Aronsen & Gulden. The taxon is herein proposed as a new species.

Materials & methods

The description is based on six collections from two different years and two different localities including specimens in all stages of age. Preliminary observations and photos were made in daylight in the field, while more thorough observations and descriptions were made the same day in lamp light in the laboratory of the research centre. The dried material was rehydrated in 2% KOH and examined in ammoniated Congo Red and Melzer's reagent using a Nikon light microscope with high resolution, 100x oil objective. For each collection 30 spores were measured. The spore length and width ratios (q) were calculated and the average quotient value (q_{av}) is given in the description. Measurements and drawings were made of basidia, spores, cheilocystidia, pileipellis hyphae, and the stipe cortical layer. Melzer's reagent was used to check amyloidity of spore walls and colour reactions in the lamellar trama. Authors abbreviations follow Index Fungorum (2011), and the collections are deposited in the university museum of Oslo (O).

To assess the phylogenetic position of *M. guldeniana* within *Mycena*, and the relationship of this taxon to the morphologically similar *M. terena*, sequences of the 5′ end of the nuclear large ribosomal subunit gene (nucLSU) spanning domains D1 and D2 were analyzed within a broader sampling of taxa representative of *Mycena* and the tricholomatoid clade sensu Matheny et al. (2006). Two members of *Boletales* were included as outgroup taxa for rooting purposes. Genomic DNA was extracted from dried basidiomes representing each taxon (Table 1) using the E.Z.N.A. Forensic DNA Kit (Omega Bio-Tek, U.S.A.) according to the manufacturer's protocols. PCR protocols followed Perry & Pfister (2007) using primers LROR and LR5 (Moncalvo et al. 2007). Amplified DNA was cleaned with ExoSAP-IT (USB Molecular Biology Reagents and Biochemical, U.S.A.) and sent to Elim Biopharmaceuticals (http://www.elimbiopharm.com/) for cycle sequencing and visualization on an ABI 3700 capillary sequencer (Applied Biosystems, U.S.A.) using the same primers as above. Sequences were edited and assembled using Sequencher 4.0 (Gene Codes Corp., U.S.A.). The resulting data matrix was aligned manually with MacClade 4 (Maddison & Maddison, 2000). Edited sequences have been deposited in GenBank (Table 1), and the aligned dataset is available via TreeBase (www.treebase.org; S11538).

Maximum likelihood (ML) analyses were conducted in PAUP* version 4 (Swofford 2003) and employed an iterative approach. Starting trees were built via the Neighbor Joining method and used to estimate parameters of the model of sequence evolution. Estimated parameter values were then fixed, and a ML search was conducted. Resulting topologies were then used to re-estimate and fix parameter values. This process was repeated for a total of three iterations to insure the analyses had converged on the most likely topology. Clade support was assessed by non-parametric, maximum likelihood

Table 1. List of sequences included in this study and associated GenBank accession numbers.

Species	Collection	GenBank Accession #
Asterophora lycoperdoides (Bull.) Ditmar	CBS 170.86	AF223190
Boletellus projectellus (Murrill) Singer	MB 03-118	NG_027638
Clitocybe dealbata (Sowerby) Gillet	HC 95.cp3	AF223175
Collybia tuberosa (Bull.) P. Kumm.	TENN53540	NG_027631
Entoloma prunuloides (Fr.) Quél.	TJB4765	AY700180
Mycena clavicularis (Fr.) Gillet	RV87/6	AF042637
Mycena crocata (Schrad.) P. Kumm.	BAYER G 057	AY207241
Mycena galericulata (Scop.) Gray	GLM 45970	AY207251
Mycena guldeniana	**A 36/05**	**JF944831**
Mycena haematopus (Pers.) P. Kumm.	HKI ST 22545	AY207252
Mycena insignis A.H.Sm.	DAOM208539	AF261413
Mycena leaiana (Berk.) Sacc.	DAOM167618	AF261411
Mycena maculata P. Karst	GLM 4597	AY207254
Mycena niveipes (Murrill) Murrill	GLM 45975	AY207242
Mycena olivaceomarginata (Massee) Massee	GLM 45976	AY207255
Mycena plumbea P. Karst.	PBM 2718	DQ470813
Mycena polygramma (Bull.) Gray	GLM 45977	AY207243
Mycena pura (Pers.) P. Kumm.	JM98/136	AF261410
Mycena renati Quél.	GLM 45979	AY207256
Mycena rubromarginata (Fr.) P. Kumm.	GLM 45981	AY207245
Mycena sanguinolenta (Alb. & Schwein.) P. Kumm.	GLM 45982	AY207257
Mycena terena	A 41/09	JF944832
Mycena tintinnabulum (Fr.) Quél.	GLM 4598	AY207258
Mycena viscidocruenta Cleland	DUKE3411	AF261414
Mycena zephirus (Fr.) P. Kumm.	GLM 45984	AY207259
Suillus pictus (Peck) A.H. Sm. & Thiers	MB03-002	AY684154
Termitomyces sp.	ZA164	DQ110875
Tricholoma aestuans (Fr.) Gillet	PBM2494	AY700197

bootstrap analyses (Felsenstein 1985) as implemented in GARLI (Zwickl 2006), and consisted of 1000 replicates with all parameter values estimated by the program. All analyses were performed under a GTR+I+G model of sequence evolution as determined using the Akaike Information Criterion in MrModelTest 2.3 (Nylander 2004). Bayesian

analyses were performed using Metropolis-coupled Markov Chain Monte Carlo (MCMCMC) methods as implemented in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) using the same model as the ML analyses. Analyses consisted of two parallel searches, run for 3 million generations and initiated with random starting trees. Chains were sampled every 300 generations for a total of 10,000 trees per search, sampled from the posterior distribution. Trees sampled prior to the analysis reaching a split deviation frequency of 0.02 were discarded as the burn-in, while the remaining trees were used to calculate the posterior probabilities of the individual clades. Default settings in MrBayes were used to set the incremental heating scheme, chain number, unconstrained branch lengths and uninformative topology priors.

Taxonomy

Mycena guldeniana Aronsen & B.A. Perry, **sp. nov.** FIG. 1–7

MYCOBANK MB 561558

Basidiomata solitaria vel gregaria. Pileus usque ad 2 mm latus, hemisphericus, conicus vel parabolicus, interdum papillatus, plerumque sulcatus, translucente striatus, pruinosus, glabrescens, pallide brunneus, pallide griseo-brunneus vel obscure griseus, centro obscurior. Caro tenuis, pallida, odore nullo. Lamellae 4–8 stipitem attingentes, adnatae vel late adnatae, pallide brunneae, pallide griseae vel albae. Stipes usque 30 × 0.2 mm, brunneo-griseus vel griseus deorsum albido-pallescens, basi fibrillis albis instructus.

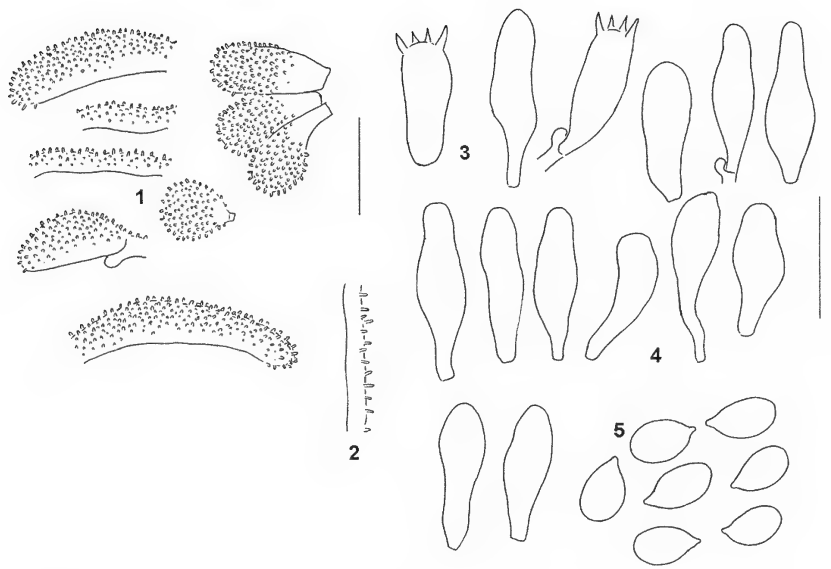
Basidia 18–33 × 7–11 µm, clavata, (2–)4-spora, fibulata. Sporae 7.5–8.7–10 × 4.8–5.5–6.5 µm, amyloideae. Cheilocystidia 21–40 × 6–11 µm, cylindracea, subclavata, sublageniformia, fibulata, laevia. Pleurocystidia nulla. Hyphae pileipellis 5–10(–17) µm latae, fibulatae, diverticulatae, cellulae terminales usque 15 µm latae, clavatae vel subcylindraceae, diverticulatae. Hyphae stipitis corticales 1–7 µm latae, fibulatae, diverticulatae.

Ad Salicis *folia decisa, in zona alpina.*

TYPE: NORWAY, Telemark, Tinn, Gaustakneet, UTM(WGS84): MM 8249 3560, altitude ca. 1150 m., 21 Sept. 2008, leg. A. Aronsen A54/08 (**HOLOTYPE** O-F300034).

ETYMOLOGY: The species is named after Prof. Gro Gulden, Oslo, in recognition of her contributions to the knowledge of arctic and alpine fungi.

Pileus 1–2 mm across, hemispherical, then conical to parabolical, flattening to convex, becoming more or less plano-convex with or without a small papilla centrally, occasionally somewhat depressed centrally, pruinose, glabrescent, shallowly sulcate, more or less translucent-striate, pale brown or pale grey-brown or grey to dark grey, sometimes pale grey, the centre dark brown to dark grey. Flesh very thin, whitish. Odour and taste indistinctive. Lamellae 4–8 reaching the stipe, with or without lamellulae, usually well developed and fairly broad, but occasionally only showing as faint ridges, ascending, the edge concave to convex, narrowly adnate to broadly adnate, sometimes decurrent with a very short tooth, pale brown, pale grey to white, the edge pallid. Stipe up to 30 × 0.2 mm, very thin, hollow, equal, terete, firm, pruinose, glabrescent, becoming shiny, curved to flexuous, brownish grey to watery grey, becoming more whitish; sometimes dark grey at the apex in younger specimens; often



FIGURES 1–5. *Mycena guldeniana* (holotype). 1. Hyphae of the pileipellis with terminal cells. 2. Hypha of the cortical layer of the stipe. 3. Basidia. 4. Cheilocystidia. 5. Spores. Bar = 20 µm.

conspicuously insititious but occasionally attached to the substratum by a whorl of radiating, flexuous, white mycelial fibrils.

Basidia 18–33 × 7–11 µm, clavate, (2- and) 4-spored, with plump sterigmata up to 5 µm long. Spores 7.5–8.7–10 × 4.8–5.5–6.5 µm, q = 1.3–1.9, q_{av} ~ 1.6, broadly ellipsoid to pip-shaped, smooth, amyloid. Cheilocystidia 21–40 × 6–11 µm, occuring mixed with the basidia, cylindrical, subclavate, sublageniform, smooth. Pleurocystidia absent. Lamellar trama dextrinoid, reddish brown in Melzer's reagent. Hyphae of the pileipellis 5–10(–17) µm wide, densely covered with cylindrical, straight excrescences 1–3.5 × 0.5 µm; terminal cells up to 40 × 15 µm, clavate to subcylindrical, covered with short, straight, cylindrical excrescences. Hypodermium of broad, cylindrical to subglobose, smooth cells 15–40 × 13–28 µm. Hyphae of the cortical layer of the stipe 1–7 µm wide, densely covered with cylindrical, sometimes more thorn-like, straight excrescences 1–6 × 0.5–1 µm; terminal cells not typically differentiated but a few observed at the base of the stipe being clavate, 26–35 × 5–10 µm, densely covered with cylindrical excrescences 1–4 × 0.5–1 µm. Clamp connections observed at the septa of some of the basidia, the cheilocystidia, and the hyphae of the pileipellis and the stipe cortex.

Solitary or in small groups on fallen, decaying leaves of *Salix* sp.; occasionally on small, fallen twigs on the ground in dense *Salix* shrubs.

Additional specimens examined — **NORWAY**: **Hordaland**, Ulvik, Finse, Nordnut sør, UTM(WGS84): MN 1667 1903, alt. ca. 1400 m, 27 Aug. 2005, leg. A. Aronsen A 34/05 and A 36/05, GenBank JF944831; 23 Aug. 2008, leg. A. Aronsen A 30/08; **Telemark**, Tinn, Gaustakneet, UTM(WGS84): MM 8249 3560, alt. ca 1150 m, 14 Sept. 2008, leg. A. Aronsen A 44/08; 21 Sept. 2008, leg. A. Aronsen A 63/08.

Discussion

Mycena guldeniana belongs to section *Polyadelphia* Singer ex Maas Geest. (Maas Geesteranus 1986) based on its small size, adnate lamellae, pileipellis and stipitipellis hyphae densely covered with short cylindrical excrescences, absence of pleurocystidia, and occurrence on decaying leaves. The species of this section usually possess cheilocystidia that are densely covered with short, cylindrical excrescences, but Aronsen & Maas Geesteranus (1992) described the new species *M. terena* with smooth cheilocystidia. Another species in the same section known to have more or less smooth cheilocystidia is *M. querciphila* Esteve-Rav. & M. Villarreal (Esteve-Raventós & Villarreal 1997).

The smooth cheilocystidia of *M. guldeniana* suggest a close relationship with both *M. terena* and *M. querciphila*, with *M. terena* seeming the most similar. *Mycena querciphila* differs in the pale olive-yellow to olive-brown colours of pileus, lamellae, and stipe and in having some cheilocystidia sparsely diverticulate by warts or short cylindrical excrescences. Additionally, *M. querciphila* is typically found growing on fallen, decaying leaves of *Quercus ilex* subsp. *ballota*. *Mycena terena*, recorded on fallen decaying *Salix caprea* leaves in two coastal lowland localities in South Norway, has a pale beige or very pale grey pileus that soon turns white, a black stipe apex (when young), somewhat narrower spores, and pileipellis hyphae that lack prominent terminal cells. The differences are shown in Table 2 below.

Table 2. A comparison between *Mycena guldeniana* and *M. terena*.

	Mycena guldeniana	*Mycena terena*
Pileus	pale brown to grey-brown	pale beige or pale grey, soon fading to white
Stipe apex	white to dark grey in young specimens	black in young specimens
Spores	q_{av} ~1.6	q_{av} ~ 1.8
Hyphae of the pileipellis	with prominent, inflated terminal cells	without prominent terminal cells
Habitat	on fallen leaves and twigs under *Salix* spp. in alpine area	on fallen leaves of *Salix caprea* in coastal lowland

FIGURE 6. *Mycena guldeniana* (holotype).

On 11 Aug. 1979, Gulden & Jenssen (1982) reported an unknown species of *Mycena*, collected at Finse, Nordnut, on a dead leaf. Although their material was 'too scanty for a formal description of a new species,' the description indicates that their unknown species was identical with *M. guldeniana*.

Phylogenetic analyses

The nucLSU dataset comprises 887 aligned positions for 26 ingroup taxa and contains 131 parsimony informative characters. Maximum likelihood analyses converged on a common topology, with each iteration producing trees that did not differ significantly in their likelihood values. The final ML tree recovered is shown in FIGURE 7 (–*ln*L = 3527.88466). Bayesian analyses reached a split deviation frequency below 0.02 after approximately 880,000 generations, and the first 2930 trees sampled were discarded as the burn-in. All *Mycena* sequences included in the analyses form a well-supported (ML bootstrap = 100, posterior probability = 1.0) lineage, sister to the remaining taxa sampled from the tricholomatoid clade. Although relationships within the *Mycena* lineage are not resolved with significant support, *M. guldeniana* and *M. terena* are

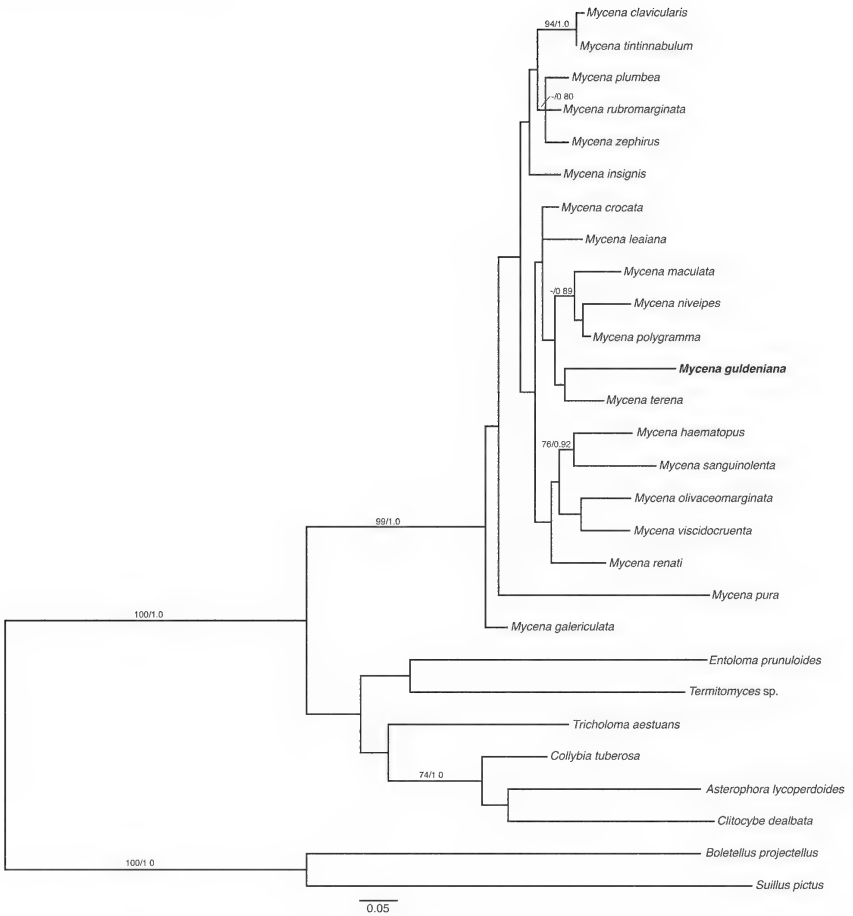


FIGURE 7. Maximum likelihood topology of *Mycena guldeniana* and other *Mycena* species inferred from nucLSU sequence data (–lnL = 3527.88466). Numbers separated by / represent maximum likelihood bootstrap proportions and bayesian posterior probabilities greater than 70% and 0.70, respectively (– designates a value below 70% / 0.70).

resolved as sister taxa in all topologies recovered by the ML analysis. Although the relationship between these taxa is not well supported by ML bootstrap or bayesian posterior probabilities, these results do support our recognition of *M. terena* and *M. guldeniana* as distinct phylogenetic species. While these two taxa are very similar morphologically, the nucLSU data indicate that genetically they are as distinct from one another as are the majority of other closely related *Mycena* species included in our analyses.

Acknowledgments

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Neolecta vitellina, first record from Romania, with notes on habitat and phenology

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Abstract — *Neolecta vitellina,* one of the rarely collected ascomycete species in Europe, is reported from Romania for the first time. The species was found on the ground, under Norway spruce (*Picea abies*) in 2004, from the Nature Reserve "Tinovul Mare" Poiana Stampei (Eastern Carpathians, Romania). Subsequent field observations have confirmed the presence of *Neolecta vitellina* in the same location, in the period 2005–10. A description and photographs of the specimens are presented.

Key words — *Ascomycota, Neolectaceae,* ITS sequence

Introduction

The genus *Neolecta* Speg. belongs to the ascomycete family *Neolectaceae* (Redhead 1977), order *Neolectales* (Landvik et al. 1993), class *Neolectomycetes* (*Taphrinomycotina, Ascomycota*). This genus includes three accepted species —*N. flavovirescens* Speg., *N. irregularis* (Peck) Korf & J.K. Rogers, *N. vitellina*— with clavate, unbranched to lobed yellow ascomata, up to about 7 cm tall (Landvik et al. 2003). Of these three species, only *N. vitellina* has been reported from Europe (Bresadola 1882, Geitler 1958, Hansen & Knudsen 2000: 48–50, Krieglsteiner 1993: 421, Landvik et al. 2003, Ohenoja 1975, Redhead 1989). Work published by European researchers about *Neolecta vitellina* refers mainly to phylogeny, morphology and ultrastructure (Landvik 1996, 1998; Landvik et al. 1993, 2001, 2003). The purpose of this paper is to report the presence of *Neolecta vitellina* in Romania and to provide morphological and ecological notes on this fungus.

Materials & methods

During 2005–2010 the known habitat of *Neolecta vitellina* in the Nature Reserve "Tinovul Mare" Poiana Stampei (Eastern Carpathians, Romania) was monitored for

Table 1. List of *Neolecta vitellina* specimens, GenBank (ITS) accession numbers, and sequence identity compared with the Romanian specimen.

Herbarium: Voucher No.	Accession No.	Sequence identity with FJ171855.1	Origin	Date; Collector(s)
I 114506	FJ171855.1	N/A	Romania	2 July 2005; VC Chinan
FH:DAH-7	FJ171850.1	99%	Norway, near Oslo	14 Aug 2002; S Landvik, DA Hewitt, P Inderbitzin, S Huhtinen, inter al.
FH:DAH-18	FJ171849.1	99%	USA, MA	3 Nov 2005; DA Hewitt, B Wolfe
FH:DAH-22	FJ171847.1	99%	USA, MA	15 Nov 2005; WJ Neill
FH:DAH-21A	FJ171852.1	99%	USA, MA	17 Nov 2005; DA Hewitt
FH:DAH-21B	FJ171853.1	99%	USA, MA	17 Nov 2005; DA Hewitt
FH:DAH-11	FJ171851.1	99%	USA, MA	6 Nov 2003; DA Hewitt, G Riner
FH:KH.04.34	FJ171848.1	100%	USA, NM	30 Aug 2004; K Hansen, B Perry
OSC:119159	FJ171854.1	99%	USA, OR	3 Oct 2001; M Russell

ascomata. Fresh specimens were photographed in situ prior to collection. Ascomata were sectioned and mounted in Melzer's reagent for observation of asci and ascospores under the light microscope.

The ITS DNA sequence was generated from a dried *Neolecta vitellina* ascoma (I 114506) that was crushed using a FASTPREP DNA extraction machine (Qbiogene, Inc.). DNA was extracted from crushed material using a phenol-chloroform extraction protocol. The ITS (Internal Transcribed Spacer) region was amplified by PCR using primers ITS4 and ITS5 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). We used BlastN (Altschul et al. 1997) to query the GenBank database for similar sequences and calculate percent identity and gaps. The obtained ITS sequence is deposited in GenBank (accession number FJ171855.1).

Analyzed specimens are deposited in the Herbarium of Alexandru Ioan Cuza University, Faculty of Biology, Iaşi, Romania (I) and Farlow Herbarium, Harvard University, Cambridge, USA (FH).

Taxonomy

Neolecta vitellina (Bres.) Korf & J.K. Rogers, Phytologia 21: 204 (1971). Fig. 1

≡ *Geoglossum vitellinum* Bres., Rev. Mycol. 4: 212 (1882).

Ascomata 20–40 mm long, irregularly clavate, lanceolate or spathulate and consisting of a sterile zone (stipe) at the bottom and a fertile zone (hymenium)

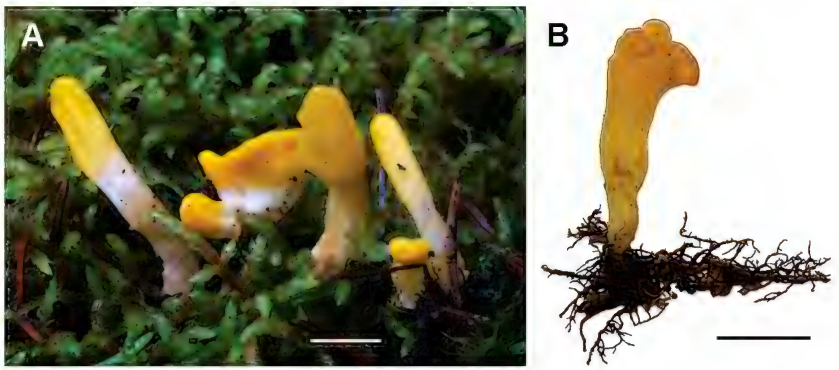

FIGURE 1. *Neolecta vitellina.*
A-ascomata in habitat; B-ascoma attached to a root of Norway spruce (*Picea abies*).
Scale bar = 1 cm.

on the top; fertile zone 9–18 mm long, 3–8 mm wide, yellow to bright yellowish, smooth, sometimes longitudinally subplicate, with margin entire or slightly irregularly lobate; sterile zone 10–22 mm long, 2–4 mm wide at the fertile zone, pubescent or tomentose, whitish or pale yellow (FIG. 1A).

ASCI 55–75 µm long, 4–5.5 µm wide above, 3–3.5 µm wide below, cylindrical to cylindrical-clavate, 8-spored. Paraphyses absent. Ascospores uniseriate, unicellular, 5.5–8 × 3–4 µm, reniform, ellipsoid or ovoid, hyaline, smooth. Phialoconidia not found associated with ascospores.

HABITAT on the ground, among mosses, in coniferous forest under Norway spruce (*Picea abies* (L.) H. Karst.) at an altitude of 915 m.

SEQUENCE ANALYSIS BlastN searches of GenBank with the ITS sequence (646 nucleotides) of the Romanian *N. vitellina* specimen (I 114506) produced significant alignments with ITS sequences from *N. vitellina* specimens collected from Norway and USA (TABLE 1).

SPECIMENS EXAMINED: **ROMANIA**. EASTERN CARPATHIANS: Nature Reserve "Tinovul Mare" Poiana Stampei, 47°18′02.69″N, 25°06′45.59″E, 915 m altitude, on ground under *Picea abies*, 4 October 2004, leg. Chinan (I 114507); 2 July 2005, leg. Chinan (I 114506, FH:DAH-31, GenBank ITS— FJ171855.1); 29 June 2006, leg. Chinan (I 114508); 30 June 2007, leg. Chinan (I 137103); 12 July 2008, leg. Chinan (I 137104); 8 July 2010, leg. Chinan (I 137105).

Discussion

Neolecta vitellina is rarely collected in Europe, where it has a boreal-montane distribution. The species has previously been reported in Norway, Sweden, Finland (Hansen & Knudsen 2000), and Italy (Bresadola 1882, Geitler 1958). In Romania, *N. vitellina* was found in the northern part of the country at the periphery of "Tinovul Mare" Poiana Stampei peat bog on the ground among

mosses under Norway spruce (*Picea abies*). Our report represents the first record of this species for Romania and the Carpathian Mountains.

Two ascomata were first found on October 2004. In July 2005, more specimens were in the same location with 53 ascomata inventoried in approximately a 10 m^2 area. Subsequent monitoring during 2006–10 has confirmed its presence in the same place and shown that late June and early July seem to be the optimal time for *Neolecta vitellina* to form ascomata in Romania. For comparison, Landvik et al. (2003) reported *N. vitellina* from Northern Europe in August while Redhead (1977) reports the species from North America in August–October. Additionally, a Bresadola collection of *Geoglossum vitellinum* from Southern Tyrol (Italy) in the Patouillard collection at the Farlow Herbarium is dated 'Aug. 1889', later than the fruiting time recorded herein for the Romanian populations.

The macroscopic and microscopic characters of the specimens collected from Romania are in accordance with the literature (Redhead 1977, Hansen & Knudsen 2000, Landvik 2003) and the ITS sequence derived from one ascoma (I 114506) matches those of other specimens identified as *Neolecta vitellina*.

We also confirm that *Neolecta vitellina* ascomata are attached to Norway spruce roots (Fig. 1B), on which was observed a whitish sleeve consisting of mycelium. Regarding this aspect, Redhead (1979) mentions that *Neolecta vitellina* is possibly a root parasite, based on morphology and the close association of the hyphae and the roots to which the hyphae are attached.

The presence of this species in the Nature Reserve "Tinovul Mare" Poiana Stampei, part of the European Ecological Natura 2000 Network, emphasizes the importance of this site for macrofungi conservation.

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Epitypification, morphology, and phylogeny of *Tothia fuscella*

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Abstract — The holotype of *Tothia fuscella* has been re-examined and is re-described and illustrated. An identical fresh specimen from Austria is used to designate an epitype with herbarium material and a living culture. Sequence analyses show *T. fuscella* to be most closely related to *Venturiaceae* and not *Microthyriaceae*, to which it was previously referred.

Key words — *Dothideomycetes*, molecular phylogeny, taxonomy

Introduction

We have been re-describing and illustrating the generic types of *Dothideomycetes* (Zhang et al. 2008, 2009, Wu et al. 2010, 2011, Li et al. 2011) and have tried where possible to obtain fresh specimens for epitypification and use molecular analyses to provide a natural classification.

Our previous studies of genera in the *Microthyriaceae*, a poorly known family within the *Dothideomycetes,* have resulted in several advances (Wu et al. 2010, 2011). The *Microthyriaceae* are characterized by superficial, flattened, dimidiate ascomata with cells of the upper wall that radiate and open by an ostiole. Asci are bitunicate and fissitunicate and ascospores are hyaline to brown (Hawksworth et al. 1995, Kirk et al. 2008).

Tothia is a poorly known monotypic genus forming thyriothecia currently classified in the *Microthyriaceae* (Lumbsch & Huhndorf 2010). To reassess the morphology of *T. fuscella*, we examined its holotype and re-collected the species

from stalks of *Teucrium chamaedrys* in two different regions of Austria. Cultures of these fresh collections were prepared to extract DNA and to sequence LSU and ITS rDNA in order to establish the phylogenetic position of this fungus. In this paper, we provide a detailed description and illustrations of *T. fuscella* and establish its familial placement through nuLSU rDNA sequence analyses.

Materials & methods

Type material of *Tothia fuscella* was borrowed from URM. Ascomata were rehydrated in 3% KOH prior to examination and sectioning. Specimens were examined under a Leica MZ16A stereomicroscope, and fine forceps were used to remove one or two ascomata, which were mounted in water, Melzer's, Congo red, or cotton blue reagents. Observations and photographs were made under a Nikon E800 or Nikon 80i light microscope. For some hyaline structures differential interference contrast microscopy was used. Sections were cut by hand with a sharp razor blade or into 8-µm thick slices using a Leica CM1100 freezing microtome and then were transferred to a drop of water or cotton blue for examination and photography. Measurement ranges cover a minimum of 30 ascospores, 25 asci, or 10 ascomata. Microtome sections were made from one or two ascomata, and ten measurements were made from whole ascoma and peridia.

Fresh specimens were collected in Austria and herbarium material is deposited in IFRD and WU. Single ascospore isolates were obtained following Choi et al. (1999) and grown on 2% malt extract agar (MEA). Cultures are deposited at the CBS.

Total DNA was extracted from liquid cultures according to Voglmayr & Jaklitsch (2011). A ca. 1600 bp fragment containing partial SSU, ITS1, 5.8S, ITS2 and partial LSU rDNA was amplified with primers V9G (de Hoog & Gerrits van den Ende 1998) and LR5 (Vilgalys & Hester 1990). PCR products were purified by an enzymatic PCR cleanup as described in Voglmayr & Jaklitsch (2008). DNA was cycle-sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington) with primers V9G, LR3 (Vilgalys & Hester 1990) and LR5, and an automated DNA sequencer (ABI 377 or 3130xl Genetic Analyzer, Applied Biosystems). The GenBank accession numbers of the sequences obtained in the present study are listed in Table 1.

One *Tothia fuscella* sequence was aligned with 12 sequences downloaded from GenBank (Table 1). Preliminary multiple alignments were generated using BioEdit (Hall 1999) and ClustalX v. 1.83 (Thompson et al. 1997) and then checked visually and manually optimized.

The alignment was analyzed using PAUP* 4.0b10 (Swofford 2002) and MrBayes v. 3.0b4 (Ronquist & Huelsenbeck 2003). *Schismatomma decolorans* (*Arthoniomycetes*), which is basal to other ascomycetes in a recent study (Schoch et al. 2009), was selected as outgroup.

A Maximum Parsimony (MP) tree was inferred with PAUP* using the heuristic search option with 10000 random taxa addition and tree bisection and reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and of equal weight; gaps were treated as missing data. Maxtrees were unlimited, zero-length branches were collapsed, and all most parsimonious trees were saved. Maximum parsimony bootstrap

TABLE 1. *Dothideomycetes* and *Arthoniomycetes* specimens analysed (Newly deposited sequences shown in bold).

SPECIES	CULTURE	FAMILY (Schoch et al. 2009)	GENBANK ACCESSION nuLSU rDNA
Apiosporina collinsii (Schwein.) Höhn.	CBS118973	*Venturiaceae*	GU301798
Apiosporina morbosa (Schwein.) Arx.	dimosp	*Venturiaceae*	EF114694
Dothidea insculpta Wallr.	CBS189.58	*Dothideacae*	DQ247802
Dothidea sambuci (Pers.) Fr.	DAOM231303	*Dothideacae*	AY544681
Microthyrium microscopicum Desm.	CBS115976	*Microthyriaceae*	GU301846
Myriangium duriaei Mont. & Berk.	CBS260.36	*Myriangiaceae*	DQ678059
Myriangium hispanicum J.B. Martínez	CBS247.33	*Myriangiaceae*	GU301854
Schismatomma decolorans (Turner & Borrer ex Sm.) Clauzade & Vězda	DUKE0047570	*Roccellaceae*	AY548815
Tothia fuscella	**(TE)**CBS130266	–	JE927786
Tothia fuscella	**(TF1)**CBS130267	-	JE927787
Trichodelitschia bisporula (P. Crouan & H. Crouan) Munk	CBS262.69	*Phaeotrichaceae*	GU348996
Trichodelitschia munkii N. Lundq.	Kruys201	*Phaeotrichaceae*	DQ384096
Venturia inaequalis (Cooke) G. Winter	CBS594.70	*Venturiaceae*	GU301879
Venturia populina (Vuill.) Fabric.	CBS256.38	*Venturiaceae*	GU323212

analyses were done with the same settings, with ten rounds of random sequence addition (Hillis & Bull 1993).

The consistency index (CI; Kluge & Farris 1969), retention index (RI; Farris 1989), and rescaled consistency index (RC; Farris 1989) were obtained from PAUP*.

For Bayesian analyses, evolution was estimated by using MrModeltest 2.2 (Nylander 2004). Posterior probabilities (Rannala & Yang 1996, Zhaxybayeva & Gogarten 2002) were determined by Markov Chain Monte Carlo sampling (BMCMC) in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001). Six simultaneous Markov chains were run for 1 million generations; trees were sampled every 100th generation, resulting in 10 001 saved trees.

The first 2000 trees were discarded and the remaining 8001 trees were used for calculating posterior probabilities in the majority rule consensus tree (Cai et al. 2006, 2008, Liu et al. 2010). The phylogenetic tree was visualized with TREE-VIEW (Page 1996).

Results

Because the SSU-ITS-LSU rDNA sequences obtained for the two *Tothia fuscella* isolates are identical, only one (1535 bp) was included in the analyses. Of the 735 nucleotides analyzed, 478 were constant and 182 variable characters were parsimony informative. Maximum parsimony analysis produced a single

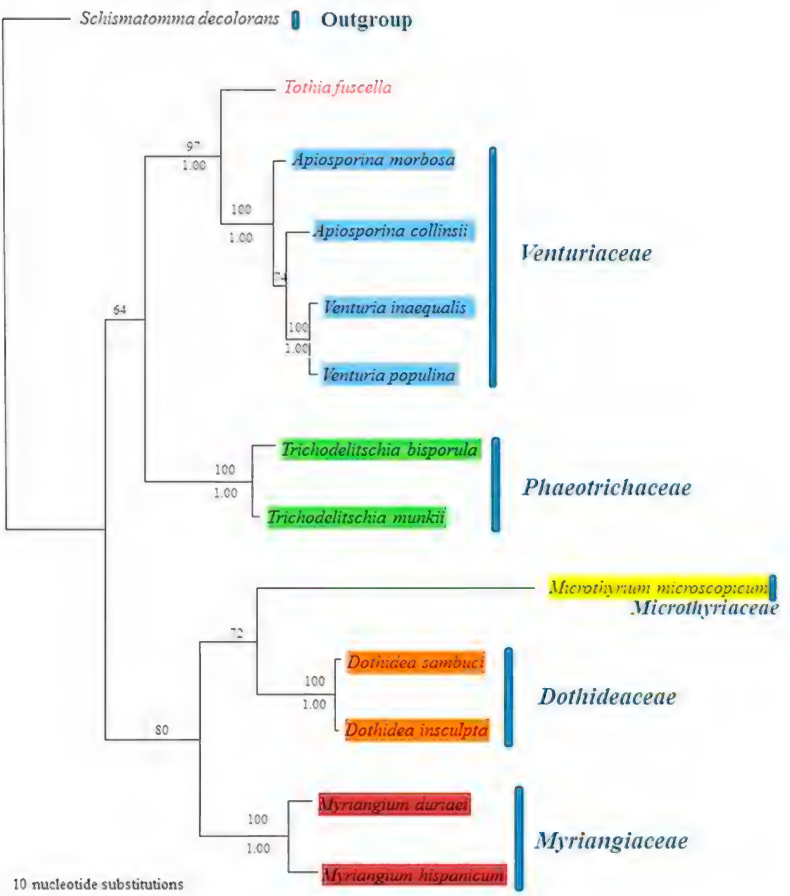

FIG. 1. Phylogram showing the single MP tree of 487 steps, inferred from a heuristic parsimony analysis of the nuLSU rDNA using PAUP*. MP bootstrap support values ≥ 50 % and Bayesian values ≥ 90 % are shown above and below branches, respectively.

MP tree of 487 steps (Fig. 1), with CI = 0.729, RI = 0.735, RC = 0.536 and HI = 0.271. The overall topology of trees sampled in the Bayesian analysis was compatible with the MP tree. MP bootstrap support values and posterior probabilities are shown in Fig. 1.

In the phylogenetic analyses (Fig. 1), *Tothia fuscella* was remote from the *Microthyriaceae* clade but clustered near the *Venturiaceae* clade with 97% bootstrap support and 1.0 posterior probability.

Taxonomy

Tothia Bat., in Toth, Annls hist.-nat. Mus. natn. hung. 52: 105 (1960).

Saprobic on stems. Ascomata, superficial, thyriothecial; in section conical, relatively small, opening with a minute flat or slightly papillate ostiole. Asci 8-spored, bitunicate, fissitunicate, cylindrical or obclavate. Ascospores, 1-septate, light brown.

Anamorphs: None reported for the genus (Hyde et al. 2011).

Tothia fuscella (Sacc.) Bat., in Toth, Annls hist.-nat. Mus. natn. hung. 52: 106 (1960). Fig. 2

≡ *Microthyrium fuscellum* Sacc., Michelia 2(6): 57 (1880).

Saprobic on stems of *Teucrium chamaedrys*. Superficial mycelium absent. Ascomata, solitary or gregarious, superficial, appearing as small black dots on the host surface, thyriothecial; in section 130–270 µm diam, 60–122 µm high, dome-shaped or flat-conical, dark brown, membranaceous, opening by a short papillate ostiole. Peridium 7–15 µm wide above, dark brown, comprising a single upper layer of cells of textura angularis; ascoma base, comprising hyaline ellipsoidal cells. Hamathecium of pseudoparaphyses 1.5–3 µm wide, longer than asci. Asci 57–71 × 9–13 µm (mean = 64 × 10.5 µm, n = 25), 8-spored, bitunicate, fissitunicate, obclavate, with a knob-like short pedicel about 4–6 × 5–7 µm, lacking an ocular chamber. Ascospores 24–28 × 4–6 µm (mean = 25.7 × 4.7 µm, n = 30), 2–3 seriate, fusiform or oblong-ellipsoid, light brown, one-septate, slightly constricted at the septum, upper cell slightly wider than the lower, with four guttules, smooth-walled.

After critical morphological comparisons showed that the fresh collections were identical with the holotype, we designated one specimen as the epitype for *T. fuscella*.

Materials examined: **AUSTRIA, Kärnten**, St. Margareten im Rosental, Aussicht, grid square 9452/3, on stalks of *Teucrium chamaedrys* (*Lamiaceae*), soc. *Ophiobolus erythrosporus*, 3 July 2010, W. Jaklitsch (**epitype designated here,** WU31396; ex-epitype culture TF1; LSU-ITS sequence JF927787; iso-epitype IFRD8982); 12 Sep 2010, W. Jaklitsch & O. Sükösd (WU 31397). **Niederösterreich**, Krems, Egelsee, on stems of *Teucrium chamaedrys*, soc. *Ophiobolus* sp., 15 Sep 2010, H. Voglmayr (WU 31398; culture TE; ITS-LSU sequence JF927786). **HUNGARY**, on stems of *Teucrium chamaedrys*, further data not given (holotype, URM 8210).

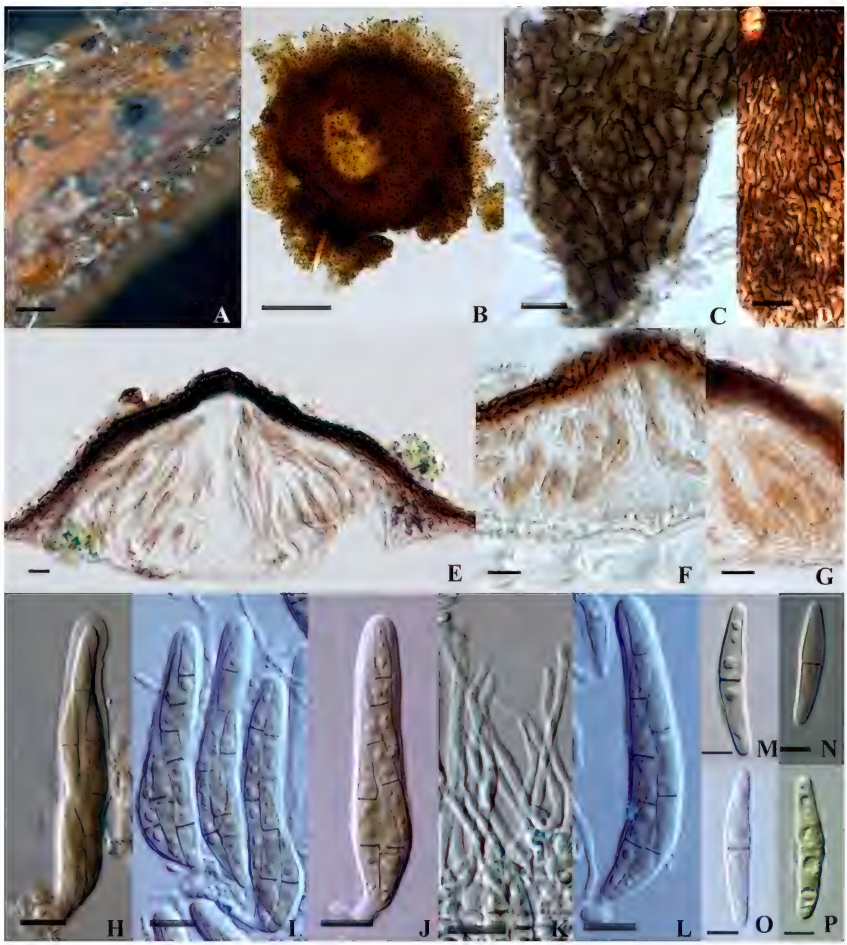

Fig. 2. *Tothia fuscella* (A, D–E, H, N from epitype; B–C, F–G, I–M, O–P, from holotype). A. Appearance of ascomata on the host surface. B, C, D. Squash mount of ascoma. E–G. Ascomata in Section. H–J, L. Asci. K. Hamathecium. M–P. Ascospores. Scale bars: A = 500μm, B = 40 μm, C–L = 10 μm. M–P = 5 μm.

Comments—In this paper we redescribe the type of *Tothia fuscella* and designate an epitype with dried material and an associated living ex-type culture. Ascomata of *T. fuscella* are morphologically indistinguishable from typical representatives of *Microthyriaceae*. Its ascospores resemble *Microthyrium* in being one-septate, guttulate, and slightly asymmetrical but differ in their dilute brown color. Phylogenetic analyses indicate that *T. fuscella* is most closely related to the *Venturiaceae*, to which it should be assigned.

Thyriothecial ascomata are unusual for the *Venturiaceae*, which illustrates that this ascoma type has evolved independently several times within *Dothideomycetes*. Genera in *Venturiaceae* usually have yellowish, greenish brown to brown, one-septate ascospores and obclavate asci (Barr 1968, 1989), which agrees well with *Tothia*.

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Exserohilum neoregeliae sp. nov., a new pathogen of *Neoregelia carolinae*

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Abstract —*Exserohilum neoregeliae* is described from *Neoregelia carolinae* leaves imported from the Netherlands at plant quarantine inspection in Japan. The new fungal species is characterized by long rostrate conidia with an obconic basal end cell with a distinct hilum. ITS + 5.8SrDNA sequence analyses revealed that *E. neoregeliae* forms a monophyletic group with *E. minor*.

Key words — hyphomycetes, *Bromeliaceae,* leaf blight, phylogeny

Introduction

Neoregelia carolinae (Beer) L.B. Sm. (*Bromeliaceae*) is an ornamental plant native to South America (Takabayashi 1994). In May 2006, brown spindle-shaped lesions were found on *N. carolinae* leaves imported from the Netherlands during plant quarantine inspection at Narita International Airport in Japan. A new species of *Exserohilum* isolated from the lesions is described based on its distinctive conidial morphology. In addition, molecular phylogenetic analyses were carried out on the internal transcribed spacer of ribosomal DNA (rDNA-ITS), including 5.8S rDNA.

Materials & methods

Isolates (IM201-c, -d, -e, -f) were obtained from four lesions on the leaves of *N. carolinae* and used for morphological observation, pathogenicity tests, and molecular phylogenetic analyses. For morphological observation, the isolates were incubated on V8 juice agar under alternating 12 h black light blue (BLB)/12 h darkness at 25°C for about 20 days, and the resulting anamorph was observed under light microscopy. Pathogenicity tests were conducted by placing V8 juice agar pieces, including hyphae, on needle-wounded leaves of the original host that was grown in a greenhouse for four

Table 1. Conidial morphology of *Exserohilum neoregeliae* and similar species.

Species	*neoregeliae* [a]	*longisporum* [b]	*rostratum* [c]	*longirostratum* [c]	*minor* [c]
Shape	Narrowly obclavate or rostrate	Fusiform, narrowly obclavate or rostrate	Ellipsoid, narrowly obclavate, or rostrate	Narrowing gradually to long apical beak	Fusiform
Color	Pale brown to brown	Yellowish brown	Brown or olivaceous	Olivaceous brown	Olivaceous brown
No. of distosepta	6–20 (–26)	(6–)8–13 (–18)	≤ 18	—	5–11
Length	80.5–285	54–240	15–200	60–475	55–135
Width	12.5–27	11.5–21.5	7–29	12–26	12.5–20
Septa	Concolorous	Concolorous	Darker & thicker at basal septum	End cells often cut off by dark, thick septum	Concolorous
Base	Basal cell obconic; occ. constricted at/ below septum	Basal cell often sl. bulging	Subhyaline	Broadest at base	Base truncate obconic; lower half occ. constricted

[a] = Isolate No. IM201-D; [b] = Sun et al. (1996); [c] = Sivanesan(1986)

months. The aseptic V8 juice agar pieces were placed in the same way as a control. The inoculated plants were kept in a plastic case with a cover to maintain high humidity for 2 days. Then they were kept at room temperature under natural light after uncovering. The ITS+5.8S rDNA were amplified with ITS1/4 primers (White et al. 1990), sequenced directly, and analyzed to reveal the phylogenetic relationship of the new species with other *Exserohilum* species registered in the DNA Data Bank of Japan (DDBJ; see also Berbee et al. 1999, Chung et al. 2005). The DNA sequences were aligned using ClustalX v2.0.7 (Larkin et al. 2007). Phylogenies were generated using distance methods. The distance matrix for the aligned sequences was calculated using Kimura's two-parameter method (Kimura 1980) and was analyzed with the neighbor-joining method (Saitou & Nei 1987) using ClustalX v2.0.7 (Larkin et al. 2007). The sequences of isolates IM201-D and IM201-E were registered in DDBJ as AB607956 and AB607957, respectively.

Symptoms

The brown spindle-shaped lesions were usually observed in the center of the leaves (Fig. 1A, B) but occasionally near the leaf margin (Fig. 1C). Some lesions elongated along the veins or turned grayish white with brown margins, producing leaf blight. No sporulation was detected from any lesions under natural conditions.

Taxonomy

Exserohilum neoregeliae Sakoda & Tsukib., **sp. nov.** Fig. 1E–G

MycoBank MB 561917

Coloniae in agaro 'V8 succus' cinereae vel brunneae, floccosae. Hyphae pallide brunneae, ramosae, septatae, laevis. Stromata absentia. Conidiophora singularia, erecta, vel flexuosa,

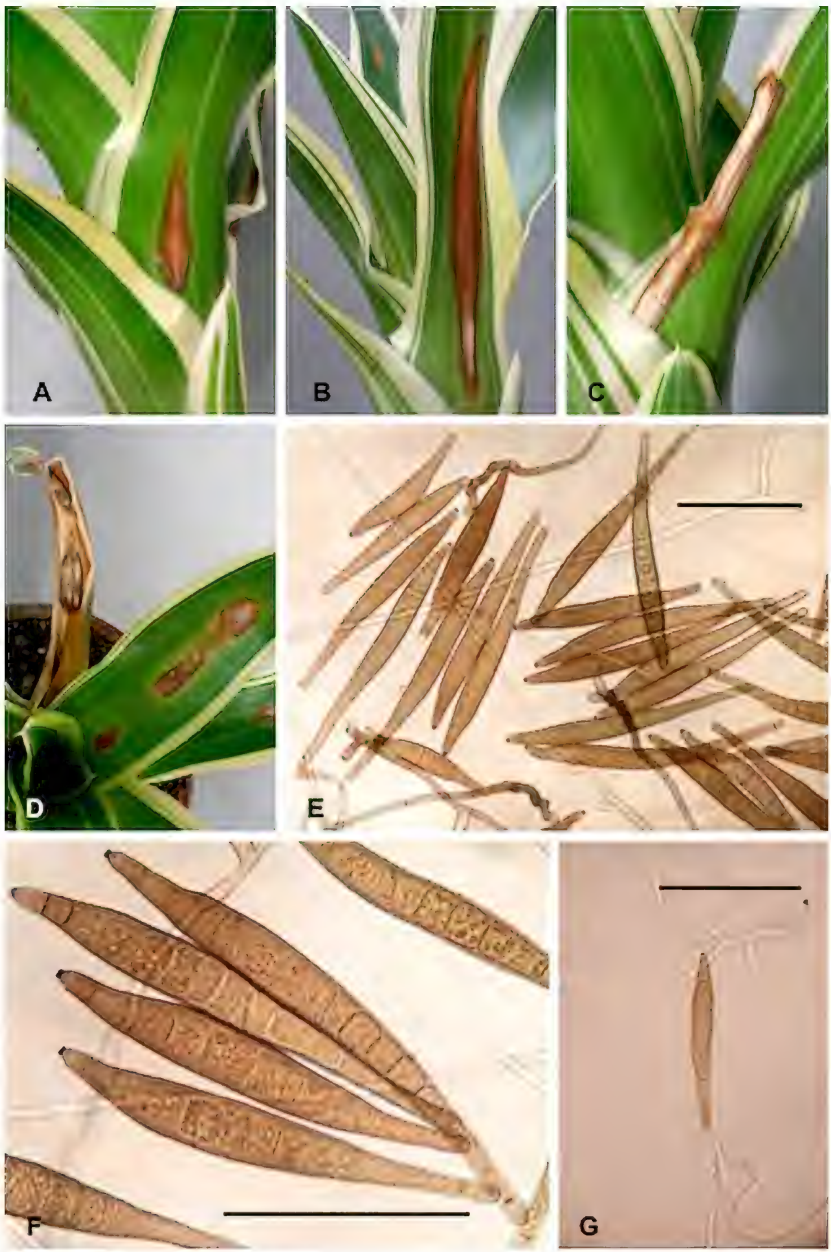

Fig. 1. *Exserohilum neoregeliae*. A–C. Natural symptoms on *Neoregelia carolinae*; D. Symptoms on *N. carolinae* inoculated with IM201-E; E–F. Conidia of IM201-D on V8 juice agar; G. Germinating conidium. Scale bars: E–G, 100 μm.

supra geniculata, laevia vel verruculosa, pallide brunnea, ad 340 µm longa, 5–10 µm lata. Conidia pallide brunnea vel brunnea, concoloria, lepto-obclavata vel rostrata, recta vel leviter curvata, laevia, ad vel infra septum basale constricta, apex obtusus, basis obconica, hilo protrudenti, 6–20(–26)-distoseptata, 80.5–285 × 12.5–27 µm. In foliis Neoregeliae carolinae.

Type: NIAESH 20605 (**Holotype** IM201-D), dry cultura isolated from affected dead leaves, Narita, Chiba, Japan, 24 May 2006, T. Sakoda, in Herbarium of the Institute of National Agro-environmental Science, Tsukuba, Japan. **Isotype:** NIAESH 20606 (IM201-E).

Colonies on V8 juice agar gray to brown, floccose. Hyphae pale brown, branched, septate, and smooth. Stromata not formed. Conidiophore solitary, straight, or flexuous, sometimes geniculate above, smooth to verruculose, pale brown, up to 340 µm long, 5–10 µm wide. Conidia pale brown to brown, concolorous, narrowly obclavate or rostrate, straight or slightly curved, smooth, sometimes constricted at basal septum or below it, apex obtuse, basal cell obconic, with a distinctly protruding hilum, 6–20(–26)-distoseptate, 80.5–285 × 12.5–27 µm (av. 187.2 × 20.6).

Key characters — The conidial morphology of *E. neoregeliae* is similar to that of *E. longisporum* G.Y. Sun (Sun et al. 1997), *E. rostratum* (Drechsler) K.J. Leonard & Suggs, *E. longirostratum* (Subram.) Sivan., and *E. minor* Alcorn (Sivanesan 1987) but does show differences (Table 1). Although concolorous and narrowly obclavate or rostrate conidia are common to both, the conidial basal end cell often bulges slightly in *E. longisporum* but is obconic in *E. neoregeliae.* While the conidial basal end cells are often cut off by thick dark septa in both *E. rostratum* and *E. longirostratum,* in *E. neoregeliae* all septa are concolorous. The conidia of *E. neoregeliae* are broader than those of *E. longisporum* and shorter than those of *E. longirostratum.* The conidia of *E. minor* are not rostrate but fusiform, although the obconic basal end cell is especially similar to that of *E. neoregeliae.*

Pathogenicity

Approximately twenty days after inoculation, symptoms similar to the natural ones were reproduced, and inoculated fungi were readily reisolated from the lesions (Fig. 1D), whereas controls remained symptom-free.

Phylogenetic analysis & discussion

According to the neighbor-joining tree derived from ITS+5.8S rDNA sequences made in this study, the two *E. neoregeliae* isolates (AB607956 and AB607957) form a monophyletic group (100% bootstrap value) close to *E. minor* (AF071341) (Fig. 2). However, the 91.6% (489/534 bp) sequence similarity between *E. neoregeliae* and *E. minor* indicates that those two species clearly differ. Moreover, despite similar conidial morphologies, *E. neoregeliae* is

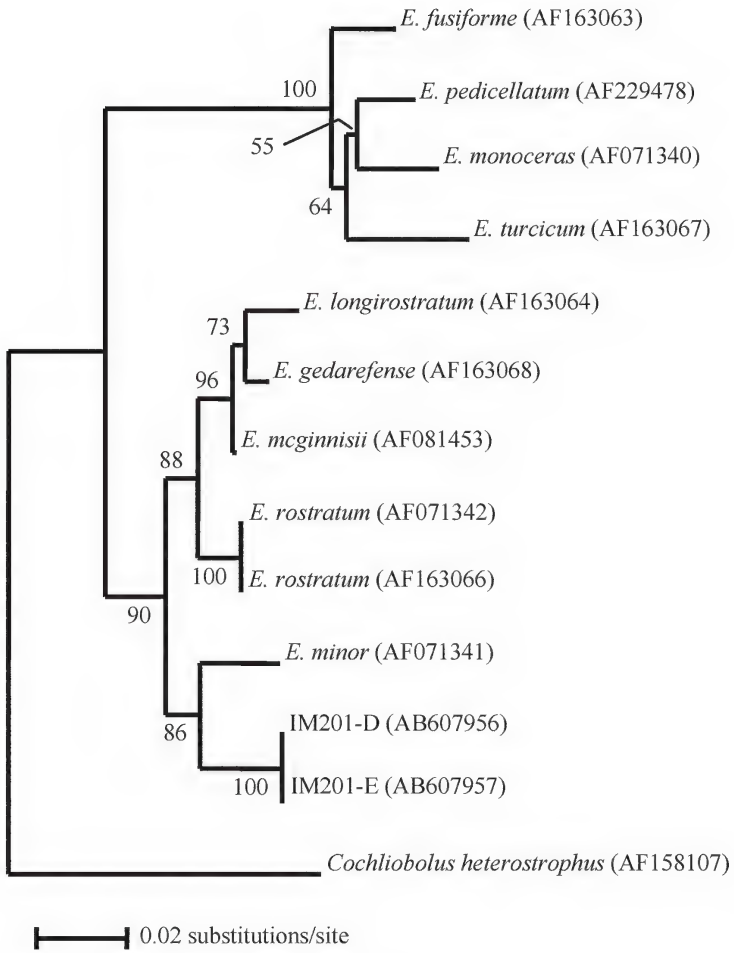

FIG. 2. A neighbor-joining tree inferred from the ITS+5.8S rDNA sequences from 10 *Exserohilum* taxa. The number in front of represented isolates shows the bootstrap values in 1000 bootstrap replicates. The rDNA-ITS accession numbers in DDBJ are shown in parentheses. The length of branches is proportional to the number of base changes, indicated by the scale bar. *Cochliobolus heterostrophus* (AF158107) is outgroup.

in a different clade from *E. rostratum* and *E. longirostratum*. Other *Exserohilum* species with relatively short or non-rostrate conidia, including *E. turcicum* (Pass.) K.J. Leonard & Suggs and *E. pedicellatum* (A.W. Henry) K.J. Leonard & Suggs (Sivanesan 1987), were considerably distant from *E. neoregeliae*. Both

morphological and phylogenetic evidence supports *E. neoregeliae* as a new species of *Exserohilum*.

Acknowledgments

The authors are grateful to Dr. Meng Zhang (Henan Agricultural University) and Dr. Wen-Hsin Chung (National Chung Hsing University) for serving as pre-submission reviewers. We also express our deep thanks to Dr. Shaun R. Pennycook (Manaaki Whenua Landcare Research) for nomenclatural review.

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Species of *Rhytismataceae* on *Camellia* spp. from the Chinese mainland

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Abstract—Six species in five different genera of the *Rhytismataceae* are reported from *Camellia* spp. on the Chinese mainland. Among them *Lophodermium sinense* on *Camellia sinensis* is described as a new species, and *Terriera camelliae* on *C. octopetala* is a new combination, while *Bifusella camelliae*, *Coccomyces sinensis*, *Hypohelion durum*, and *Lophodermium jiangnanense* are already known for China. This paper provides descriptions and a key for all these species as well as illustrations for the new species. All examined specimens are deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Key words—*Rhytismatales,* taxonomy, plant pathogens, *Theaceae*

Introduction

Members of *Camellia* L. (*Theaceae*) are evergreen shrubs or trees, with a total of 280 species distributed in bilateral zones of the Tropic of Cancer in east Asia, including 238 species in southwestern and south China (Zhang & Ren 1998). Camellias are economically valuable as ornamentals and sources of beverages, tea oil, and medicine.

Of the 1072 fungal species known to inhabit *Camellia* worldwide, only 10 belong in the *Rhytismataceae* (Farr & Rossman 2011). *Cryptomyces theae* Sawada, the first rhytismataceous species recorded on *C. sinensis* (L.) Kuntze, was described from Taiwan by Sawada (1919) and later found on the same host in Japan (Hara 1936). Minter (1982) reported *Lophodermium camelliicola* Minter on *C. sinensis* from India. Kobayashi (2007) recorded *L. hysterioides*

(Pers.) Sacc. (= *L. foliicola* (Fr.) P.F. Cannon & Minter) and two *Coccomyces* species on *C. japonica* L. in Japan. Up to now, five species of *Rhytismataceae* have been described on *Camellia* from mainland China (Teng 1933; Hou 2000; Lin et al. 2001a, 2004a,b). These fungi are mostly plant pathogens leading to different degrees of economic loss. *Lophodermium jiangnanense* can cause a leaf cast of *C. oleifera* C. Abel (Lin et al. 2004a), while *Bifusella camelliae* and *Hypohelion durum* on *C. sinensis* can cause serious branch rot (Hou 2000, Lin et al. 2004b). The present study, based on specimens collected by the authors, reports one hitherto undescribed species, makes one new combination, and discusses the four other species known on *Camellia* from the Chinese mainland. These records may not be complete, as more rhytismataceous species on camellias can be expected in this region.

Materials & methods

Macroscopic appearance was described from observations made under the dissecting microscope at 10–50 × magnification. Reference collection material was rehydrated in water for 15 min, and 10–15 µm thick sections of the fruit bodies were cut using a freezing microtome. For the observations of outlines of ascomata and conidiomata in vertical section, sections were mounted in lactic acid or cotton blue with pretreatment in water. The color of the various structures and of ascospore contents was observed in water. Gelatinous sheaths surrounding ascospores and paraphyses were examined in water or 0.1% (w/v) cotton blue in lactic acid. Measurements were made using material mounted in 5% KOH or Melzer's reagent and from 30 asci, ascospores, and paraphyses for each specimen. Line and point integrated illustrations of external shapes and internal structures of fruit bodies were drawn using a microscopic drawing tube (Panasonic XSJ-2, Japan).

Taxonomy

Bifusella camelliae C.L. Hou, Mycosystema 19: 7, 2000.

Type: **China, Anhui**, Yuexi, Wen'ao, alt. ca 1100 m, on *Camellia sinensis*, 10 May 1992, C.L. Hou 0210 (AAUF 90085).

Illustration: Hou 2000: 8, Fig. 1.

Zone lines brown to black-brown, infrequent, thin or slightly broad, sometimes not closed.

Conidiomata on twigs, scattered. In surface view conidiomata 90–190 × 100–170 µm, circular to elliptical, black-brown in the centre and at the edge of the conidioma, brown elsewhere, flattened or slightly raising the substratum surface, opening by one apical ostiole. In vertical section subcuticular. Conidia 4–6 × ca 1.2 µm, cylindrical, hyaline, aseptate.

Ascomata scattered in similar positions on the host. In surface view ascomata 500–1260 × 230–420 µm, elliptical, shinning black, edge defined, slightly raising the substratum surface, opening by a somewhat irregular

longitudinal split nearly extending to the edge of ascoma. Lips absent. In median vertical section ascomata subcuticular. Covering stroma 20–30 µm thick near the opening, gradually thinner towards the edge, connecting to the basal stroma, composed of black-brown textura globulosa-angularis with cells of 3–5 µm diam. Basal stroma 5–7 µm thick, composed of dark brown, thick-walled globular and angular cells 3–5 µm diam. Subhymenium 10–15 µm thick, consisting of hyaline textura intricata. Paraphyses slightly exceeding the asci, 1.5–2 µm wide, filiform, septate, sometimes swollen to 3–5 µm at the apex, covered with a thin mucous coating. Asci ripening sequentially, 70–100 × 12.5–14 µm, clavate, short-stalked, nearly truncate-conical to obtuse at the apex, J–, 8-spored. Ascospores biseriate or multiseriate, 19–25 × 2.5–4 µm, bifusiform, isthmus ca 1 µm wide, hyaline, aseptate, with a 0.8–2 µm thick gelatinous sheath.

Host species, habitat, and distribution: *Camellia sinensis*; producing conidiomata and ascomata in lesions on decaying and dying twigs. Known only from Anhui Province, China.

Specimens examined: On *Camellia sinensis*: **China**, **Anhui** Mt Guniujiang, alt. ca 1600 m, 2 July 2006, Y.R. Lin, S.J. Wang 2104 (AAUF 68212); Mt Tiantangzhai, alt. ca 1200 m, 23 June 2009, J.L. Chen, X.M. Gao 5294 (AAUF 71402).

Comments—*Bifusella tsugae* H.S. Cao & C.L. Hou, closely related to *B. camelliae,* differs in having ascospores with a wider isthmus, paraphyses not swollen at the apex, absence of zone lines, and occurrence on needles of *Tsuga chinensis* (Franch.) E. Pritz. (Cao et al. 1996, as *T. tchekiangensis*).

Bifusella camelliae can cause a serious branch rot of the tea plant in regions where the specimens were collected, the damage of branch tips exceeding 85% in the tea garden where the disease occurs. It may therefore pose a threat to the tea-growing industry. According to our observations, this fungus infects new twigs, gradually causing brown to grey-yellow lesions where conidiomata and ascomata are successively produced. Between May and June of the second year, ascomata mature to discharge ascospores, leading to the next infection.

Coccomyces sinensis Y.R. Lin & Z.Z. Li, Mycosystema 20: 3, 2001.

Type: **China**, **Hunan**, Changsha, Yuelushan, alt. ca 300 m, on *Camellia oleifera*, 24 June 1990, Y.R. Lin et al. 0521b (AAUF 66629b).

Illustration: Lin et al. 2001a: 4, Fig. 2.

Zone lines grey-black, thin, entirely or partly surrounding the bleached spots.

Conidiomata not observed.

Ascomata on both sides of leaves, mostly on the lower side, crowded, in subcircular bleached spots. In surface view ascomata 600–1150 µm diam., triangular to pentagonal, black-brown to black, raising the substratum surface but depressed in the central region, opening by radial splits to expose a light

orange-yellow hymenium. Lips absent. In median vertical section ascomata intraepidermal. Covering stroma 25–32 µm thick near the opening, slightly thinner towards the edge, extending to the basal stroma, black-brown, composed of 3–6 µm diam., thick-walled angular cells. Periphysoids absent. Basal stroma 12–18 µm thick, composed of dark brown textura angularis with thick-walled cells 5–8 µm diam. Excipulum arising from the inner cells of the covering stroma, 25–30 µm wide above, consisting of several rows of 2–3 µm diam., multi-septate hyphae. Internal matrix stroma 20–35 µm thick, gelatinized, consisting of loose textura intricata. Subhymenium 10–15 µm thick, composed of textura porrecta-intricata. Paraphyses filiform, 2–2.5 µm wide below, abruptly enlarged to 4–6.5 µm and subfusoid-ventricose above, with a subcylindrical, ca 1.5 µm wide pointed apex, capped with a mass of yellow-brown, subcylindrical gel 8–15 × 6–8 µm, forming a solid refractive epithecium 18–25 µm thick. Asci ripening sequentially, 110–130 × 5.5–6.5 µm, cylindrical, short-stalked, rounded at the apex, with circumapical thickening, J–, 8-spored. Ascospores fasciculate, 60–95 × 1–1.2 µm, filiform, hyaline, aseptate, covered by an inconspicuous gelatinous sheath.

Host species, habitat, and distribution: *Camellia* cf. *japonica* (Kirschner et al. 2009), *C. chekiangoleosa* Hu, *C. oleifera*; producing ascomata on dead leaves. Known from southern China and Taiwan (Kirschner et al. 2009).

Specimens examined: On *Camellia chekiangoleosa*: **China**, **Zhejiang**, Hangzhou, Liuxia, alt. ca 60 m, 6 August 1990, Y.R. Lin 0727 (AAUF 66835).

On *C. oleifera*: **China**, **Anhui**, Huangshan Arboretum, alt. ca 550 m, 5 Sep. 2009, J.L. Chen, S.J. Wang 5317 (AAUF 71425); **Fujian**, Fuzhou Arboretum, alt. ca 320 m, 2 July 1990, Y.R. Lin 0644 (AAUF 66752); **Guangdong**, Guangzhou, South China Botanical Garden, alt. ca 382 m, 22 June 1990, Y.R. Lin 0515 (AAUF 66623); **Jiangxi**, Nanchang People Park, alt. ca 25 m, 28 June 1990, Y.R. Lin 0587 (AAUF 66695).

Comments—*Coccomyces sinensis* is common on camellias in southern China and was also found recently on fallen leaves of *Camellia* cf. *japonica* in Taiwan (Kirschner et al. 2009). It is very similar in the shape of its paraphyses (subfusoid-ventricose and pointed apices) to *C. mucronatus* Korf & W.Y. Zhuang on *Fagaceae,* which differs in polygonal to subcircular ascomata, much narrower asci (4.3–5 µm) and ascospores (0.6–0.8 µm), dense periphysoids, and paraphyses lacking refractive solid gel at the apex (Korf & Zhuang 1985). *Coccomyces urceoloides* Spooner, which also somewhat resembles *C. sinensis* in paraphyses capped with a mass of subhyaline or yellowish solid gel and measuring 6–12 × 5–8 µm, differs in other aspects (Spooner 1990).

Hypohelion durum Y.R. Lin, C.L. Hou & S.J. Wang, Mycosystema 23: 169, 2004.

Type: **China**, **Anhui**, Yuexi, Wen'ao, alt. ca 1100 m, on *Camellia sinensis*, 27 May 1994, C.L. Hou 0014 (AAUF 66122).

Illustration: Lin et al. 2004b: 170, Fig. 1.

Zone lines brown to dark brown, infrequent, narrow to broad, somewhat diffused.

Conidiomata on twigs, usually crowded. In surface view conidiomata 90–230 μm diam., subcircular, black-brown in the centre and at the perimeter line, brown elsewhere, somewhat raising the substratum surface. In vertical section subcuticular. Upper wall 3–5.5 μm thick, dark brown, consisting of small angular cells. Basal wall 5–7 μm thick, black-brown. Conidiogenous cells 7.5–12 × 2–2.5 μm, flask-shape. Conidia 4.5–6.5 × 0.8–1 μm, cylindrical, hyaline, aseptate.

Ascomata in similar positions to conidiomata on the substratum, scattered to crowded in yellow-brown to grayish-white lesions. In surface view ascomata 450–950 × 260–400 μm, elliptical or broad-elliptical, black, with a clear outline, slightly raising the substratum surface, opening by a longitudinal split. Lips absent. In median vertical section ascomata subcuticular. Covering stroma 20–28 μm thick near the opening, slightly thinner towards the edge and extending to the basal stroma, composed of textura angularis-epidermoidea with black-brown or brown, thick-walled cells 4–6 μm diam. Basal stroma 12–18 μm thick, composed of 4.5–7 μm diam., black-brown, thick-walled angular and elongate cells. The triangular space between the covering and basal stroma is filled with grey-brown rather thin-walled, large angular cells. Subhymenium 18–25 μm thick, consisting of textura porrecta. Paraphyses 110–135 × 1.5–2 μm, filiform, swollen to 2.5–3.5 μm above, surrounded by a ca 1.5 μm thick gelatinous matrix. Asci ripening sequentially, 75–115 × 13–16 μm, clavate, somewhat long-stalked, apex rounded to subtruncate, J–, 8-spored. Ascospores more or less biseriate, 18–25 × 3–4 μm, cylindrical to subclavate, hyaline, aseptate, with a conspicuous gelatinous sheath 4–6 μm thick.

Host species, habitat, and distribution: *Camellia sinensis*; producing conidiomata and ascomata in lesions on decaying and dying twigs. Common in southern China.

> Specimens examined: On *Camellia sinensis*: **China**, **Anhui**, Huangshan Arboretum, alt. ca 550 m, 20 Oct. 2001, Y.R. Lin et al. 1785 (AAUF 67893); Huangshan Arboretum, alt. ca 550m, 5 Sep. 2009, J.L. Chen, S.J. Wang 5322 (AAUF 71430); **Jiangsu**, Nanjing, alt. ca 90 m, 10 April 1973, D.J. Lu 1308 (AAUF 67416).

Comments— *Hypohelion durum* is rather distinctive. It is consistent with typical features of the genus except for the presence of basal stroma and yet is far from genera such as *Hypoderma* De Not. *Hypohelion scirpinum* (DC.) P.R. Johnst. is similar but differs by its much larger ascomata (800–3000(–7000) × 500–800 μm) and ascospores with a median septum (40–75 × 4.5–6.5 μm), the absence of dark basal stroma, and its occurrence in marshes on *Scirpus* spp. (Johnston 1990). A strong parasite that is common on tea plants in southern China, *H. durum* can cause different degrees of branch rot (Lin et al. 2004b).

Lophodermium jiangnanense Y.R. Lin & S.J. Wang, Mycosystema, 23: 15, 2004.

TYPE: **CHINA, HUNAN**, Changsha, Yuelushan, alt. ca 300 m, on *C. oleifera*, 24 June 1990, Y.R. Lin 0521a (AAUF 66629a).

ILLUSTRATION: Lin et al. 2004a: 16, Fig. 2.

ZONE LINES absent.

CONIDIOMATA mostly hypophyllous, usually crowded. In surface view conidiomata 130–220 µm diam., subcircular, black-brown in the centre and at the perimeter line, brown elsewhere. In vertical section subepidermal. CONIDIA not observed.

ASCOMATA on both sides of leaves, predominantly on the lower side, crowded in grey-yellow, circular to irregular, bleached spots. In surface view ascomata 600–990 × 280–360 µm, elliptical or occasionally 3-lobed, black, slightly shiny, edge defined, raising the substratum surface, opening by a longitudinal split which is sometimes branched. Lips absent. In median vertical section ascomata subepidermal. COVERING STROMA 18–30 µm thick, extending to the basal stroma, composed of grey-brown, thick-walled, angular and elongate cells 3–3.5 µm diam., markedly black and brittle near the opening, with several rows of almost colorless, septate, cylindrical thin-walled cells occurring on the inner side of the upper margin of the covering stroma. BASAL STROMA 15–28 µm thick, composed of 4–7 µm diam., black-brown, thick-walled angular cells. The triangular space between the covering and basal stroma is filled with a hyaline, gelatinized reticulate tissue. SUBHYMENIUM 15–20 µm thick, consisting of textura porrecta. PARAPHYSES ca 1.5 µm wide, filiform, septate, often gradually swollen to 2–2.5 µm, twisted and intertwined above to form a yellow epithecium 10–15 µm thick. ASCI ripening sequentially, 95–130 × 6–7.5 µm, cylindrical, rounded at the apex, J–, 8-spored. ASCOSPORES fasciculate, 60–90 × 1.4–1.6 µm, filiform, hyaline, aseptate, with a 0.8–1 µm thick gelatinous sheath.

HOST SPECIES, HABITAT, AND DISTRIBUTION: *Camellia oleifera*; forming conidiomata and ascomata on dead part of living leaves or on fallen leaves. Known from Hunan, Anhui, and Guangdong Provinces, China.

SPECIMENS EXAMINED: On *Camellia oleifera*: **CHINA, ANHUI** Guichi, Forest Nursery of Guichi, alt. ca 600 m, 11 Oct. 1992, Q.S. Liu, Q. Cao 1640 (AAUF 67748); Huangshan, Renzipu, alt. ca 660 m, 6 Sept. 2009, J.L. Chen, Y.R. Lin 5345 (AAUF 71453); **GUANGDONG**, Guangzhou, South China Botanical Garden, alt. ca 382 m, 22 June 1990, Y.R. Lin 0515b (AAUF 66623b).

COMMENTS—*Lophodermium jiangnanense* resembles *L. intricatum* Spooner, which differs in its association with zone lines, much larger ascomata (1000–1200 × ca 500 µm) and asci (135–152 × 7–7.5µm) with truncate-conical apices, longer ascospores with a median septum, and much-branched paraphyses (Spooner 1991). This fungus usually produces fruit bodies on fallen *C. oleifera* leaves, but the collection from Anhui Province (AAUF 67748) shows that it primarily infects living leaves and develops fruit bodies in yellow-

brown to yellowish-white spots near the margin of leaves on which an obvious brown ridged zone between the healthy and sick areas has formed. It is often accompanied by *Coccomyces sinensis,* sometimes growing on the same leaves (Lin et al. 2004a).

Lophodermium sinense Y.R. Lin, C.L. Hou & Jiang L. Chen, **sp. nov.** FIGS. 1–7

MycoBank 561775

Ascomata amphigena, dispersa vel aggregata, (290–)400–730 × (150–)220–290 µm, elliptica, interdum triloba, atra, labiis carentia, ab rima longitudinali vel lobis ternis aperientia, partim subcuticularia et partim intraepidermalia, cellulis epidermalibus plus quam 4 successive in stromate basali ordinatis. Paraphyses filiformes, prope apicem plerumque gradatim tumidae et semel aut iterum ramosae, necnon epithecium formantes. Asci in successione maturescentes, 80–120 × 6–7 µm, cylindrici, brevi-stipitati, J–, 8-spori. Ascosporae 50–95 × 1.2–1.5 µm, filiformes, hyalinae, aseptatae, vagina gelatinosa 1–1.5 µm crassa indutae.

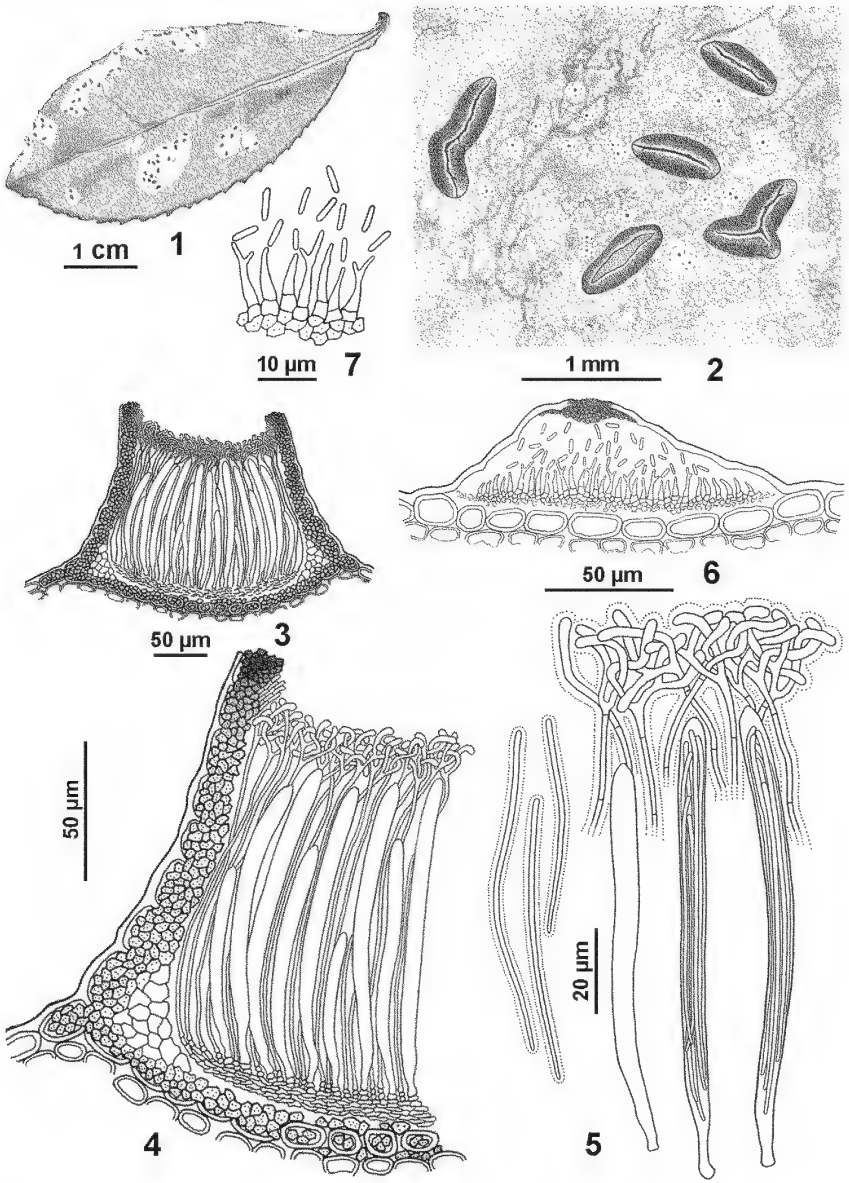
Type: China, Anhui, Mt Guniujiang, alt. ca 1600 m, on leaves of *Camellia sinensis,* 10 July 2006, Y.R. Lin, S.J. Wang 2103 (**Holotype** AAUF 68211).

Etymology: referring to the country where the specimen was collected.

Zone lines absent.

Conidiomata on both sides of leaves, scattered to crowded, occasionally merging into one another. In surface view conidiomata 80–160 µm diam., circular or subrounded, black-brown in the centre, more or less concolorous with the substratum surface elsewhere, slightly raising the leaf surface, discharging spores through a 10–15 µm diam. apical ostiole. In vertical section subcuticular. Upper wall only present around the ostiole. Basal wall absent. Subconidiogenous layer ca 7 µm thick, composed of light thin-walled angular cells. Conidiogenous cells 6–9 × 2–3 µm, cylindrical, slightly tapering towards the apex, hyaline, proliferating sympodially. Conidia 4–6 × 1 µm, cylindrical, hyaline, aseptate.

Ascomata in similar positions to conidiomata on the substratum, scattered or crowded in subcircular to irregular, yellow-brown bleached spots 5–15 mm diam. In surface view ascomata (290–)400–730 × (150–)220–290 µm, elliptical, sometimes curved or 3-lobed, with a clear outline, ends rounded or obtuse, matt black except for a grey-brown area at each end, strongly raising the substratum surface, opening by a longitudinal split more than 4/5 the length of the ascoma, which is sometimes branched, to expose a light yellow-brown hymenium. Lips absent. In median vertical section ascomata subcuticular near the opening and intraepidermal in the lower part of the covering stroma, more than four epidermal cells being displaced and lying always successively on the basal stroma. Covering stroma 12–20 µm thick, black-brown, connecting to the basal stroma, composed of textura angularis-globulosa with thick-walled cells 2–5 µm diam., markedly black and brittle near the opening, with



Figs. 1–7. *Lophodermium sinense* on *Camellia sinensis*. 1. A leaf bearing fruit bodies. 2. Ascomata and conidiomata observed under a dissecting microscope. 3. Ascoma in median vertical section. 4. Detail of ascoma in median vertical section. 5. Paraphyses, asci and ascospores. 6. Conidioma in vertical section. 7. Conidiogenous cells and conidia.

4–5 rows of light thin-walled cells occurring on the inner side of the upper margin of the covering stroma. Basal stroma slightly concave or nearly flat, 10–18 μm thick, black-brown, composed of 2–3 layers of thick-walled angular cells 2.5–5.5 μm diam. The triangular space between the covering and basal stroma filled is with 5–8 μm diam., light grey-brown, slightly thick-walled angular cells. Subhymenium 10–18 μm thick, consisting of textura angularis-porrecta. Paraphyses 1–1.5 μm wide, filiform, hyaline, septate, covered in a thin mucous coating 1.5–2 μm thick, often gradually swollen to 2–3 μm and 1–2 times branched near the apex, contorted and intertwined to above form a conspicuous epithecium 12–24 μm thick. Asci ripening sequentially, 80–20 × 6–7 μm, cylindrical, short-stalked, apex rounded, thin-walled, without circumapical thickening, J–, 8-spored. Ascospores arranged fasciculately or somewhat helically, 55–95 × 1.2–1.5 μm, filiform, slightly tapering towards the base, hyaline, aseptate, with a 1–1.5 μm thick gelatinous sheath.

Host species, habitat, and distribution: *Camellia sinensis*; ascomata develop on living leaves but are seen in a mature condition only on fallen leaves. Known only from the type locality, Anhui, China.

Comments—*Lophodermium sinense* is very similar to *L. jiangnanense* on the same plant genus, but the latter has larger subepidermal ascomata and conidiomata, unbranched paraphyses, a covering stroma comprising aliform and elongate cells, and a well developed basal stroma with hyaline reticulate tissue in the corner between the covering and basal stroma (Lin et al. 2004a). *Lophodermium intricatum* is also similar to the new species but differs in its much larger ascomata, asci, and ascospores, asci with truncate-conical apices, ascospores with a median septum and without a gelatinous sheath, and the presence of zone lines (Spooner 1991). *Lophodermium implicatum* Y.R. Lin & Z.S. Xu differs in possessing intraepidermal ascomata associated with dark brown zone lines, shorter ascospores, and intraepidermal conidiomata with trichogynes (Lin et al. 2001b).

Lophodermium sinense is a pathogen causing leaf spot. The fungus at first probably develops within the living leaves but visible symptoms do not show, after which the anamorphic and teleomorphic fruit bodies sequentially appear on brown lesions of infected leaves as the vitality of the host declines. Diseased leaves become reddish-brown and fall between July and August. Mature ascomata have been observed only on fallen leaves.

Terriera camelliae (Teng) Y.R. Lin & Jiang L. Chen, **comb. nov.**

MycoBank 561774

≡ *Lophodermium camelliae* Teng, Sinensia 4: 138, 1933

≡ *Clithris camelliae* (Teng) Tehon, Mycologia 31: 675, 1939

≡ *Colpoma camelliae* (Teng) Teng, Fungi of China: 759, 1963

Type: **China, Fujian**, Fuzhou, on fallen leaves of *Camellia* sp., Teng 1904 (No.77 in the Metropolitan Museum, Academia Sinica, Nanking, China).

Illustration: Tehon 1939: 682, Fig. 6.

Zone lines grey-brown, infrequent, thin, entirely or partly surrounding the bleached spots.

Conidiomata amphigenous, crowded. In surface view conidiomata 70-130 µm diam., subcircular to irregular, flattened, black-brown in the centre and at the perimeter line of the conidioma, grey-brown elsewhere. In vertical section intraepidermal to subepidermal. Upper wall only present around the ostiole. Basal wall 8–12 µm thick, composed of brown to dark brown tissue with no obvious structure. Conidia 2.5–4 × ca 0.8 µm, cylindrical, hyaline, aseptate.

Ascomata in similar positions to conidiomata on the substratum, scattered to crowded in grey-yellow, subcircular to irregular bleached spots. In surface view ascomata 440–870(–1020) × 290–490 µm, elliptical or occasionally 3-lobed, black-brown to black, slightly shiny, edge defined, moderately raising the substratum surface, opening by a longitudinal split about 4/5-7/8 the length of the ascoma, which is sometimes branched. Lips absent. In median vertical section ascomata subepidermal. Covering stroma 15–22 µm thick near the opening, slightly thinner towards the edge, extending to the basal stroma, composed of black-brown, thick-walled angular cells 3–5 µm diam. Along the edge of the ascomatal opening is a 10–15 µm thick, flattened extension to the covering stroma which covers the hymenium, and which comprises markedly black and brittle carbonized tissue with no obvious cellular structure. Basal stroma composed of 1–2 layers of black-brown, thick-walled, angular to globular cells. Subhymenium 12–18 µm thick, consisting of textura porrecta. Paraphyses extending 15–30 µm beyond the asci, ca 2 µm wide, filiform, sometimes gradually swollen to 2.5–3 µm or branched above, septate. Asci ripening sequentially, 85–120 × 5.5–6.5 µm, cylindrical, short-stalked, rounded at the apex, J–, 8-spored. Ascospores arranged fasciculately or somewhat helically, 52–80 × 1–1.2 µm, filiform, hyaline, aseptate, covered by a ca 0.5 µm thick gelatinous sheath.

Host species, habitat, and distribution: *Camellia octopetala* Hu, *Camellia* sp. (Teng 1933); conidiomata and ascomata were found on fallen leaves. Known only from Fujian Province, China.

Specimens examined: On *Camellia octopetala*: **CHINA, Fujian**, Fuzhou Arboretum, alt. ca 320 m, 2 July 1990, Y.R. Lin 0643 (AAUF 66751); 11 June 2009, J.L. Chen, L. Chen 5108 (AAUF 71216).

Comments—Taxonomic placement of *Terrea camelliae* has long been controversial. Teng (1933) originally validly published the fungus as *L. camelliae*. Tehon (1939), who examined part of the type (labeled "co-type" by Teng), felt that it lacked structures characteristic of the *Hypodermataceae*

(= *Rhytismataceae*) and transferred it to *Clithris* (Fr.) Bonord. (= *Cenangium* Fr.) in the *Helotiaceae* Rehm (Kirk et al. 2008). Teng's later (1963) transfer of the taxon to *Colpoma* Wallr. cannot be accepted because *Colpoma* ascomata develop on bark (not leaves) and have a well-developed basal stroma.

Eriksson (1970) erected the new genus *Terriera* B. Erikss. based on the type species *T. cladophila* (Lév.) B. Erikss. on *Vaccinium myrtillus* L. Johnston (2001) and Ortiz-García et al. (2003) transferred several species to *Terriera*, including *Clithris arundinacea* Penz. & Sacc., *C. minor* Tehon, *Lophodermium fuegianum* Speg., *L. javanicum* Penz. & Sacc., and *L. sacchari* Lyon. *Terriera* differs from *Lophodermium* Chevall. by the markedly black and brittle extensions of the covering stroma and by the thin-walled, prismatic or angular cells in the corner between the covering and basal stroma. The present species on *Camellia* sp. and *C. octopetala* fits closely the characters of *Terriera* based on the illustration by Tehon (1939) and examinations carried out by the authors. Consequently, we redispose it as *T. camelliae*.

There are some signs that *T. camelliae* may be a pathogen associated with leaf cast of eight-petaled *Camellia* species.

Key to species of *Rhytismataceae* on *Camellia* from the Chinese mainland

1a. Ascomata on twigs 2
1b. Ascomata on leaves 3
2a. Ascospores bifusiform ..... ***Bifusella camelliae***
2b. Ascospores cylindrical, elliptical or subclavate ..... ***Hypohelion durum***
3a. Ascomata triangular to pentagonal, opening by radial splits .. ***Coccomyces sinensis***
3b. Ascomata elliptical, opening by a longitudinal split 4
4a. Ascomata partly subcuticular and partly intraepidermal ***Lophodermium sinense***
4b. Ascomata subepidermal 5
5a. Covering stroma with a markedly black brittle flattened extension covering the hymenium ..... ***Terriera camelliae***
5b. Covering stroma without such an extension ..... ***Lophodermium jiangnanense***

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A new species of *Coccomyces* (*Rhytismatales*, *Ascomycota*) from Mt Huangshan, China

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Abstract—A fungus found on leaves of *Osmanthus fragrans* from Mt Huangshan in Anhui Province, China, is described as *Coccomyces minimus*. The new species is similar to *C. cyclobalanopsis* but differs in the extremely small, subepidermal ascomata and in the presence of conidiomata. The type specimen is deposited in the Forest Fungi Dried Reference Collection of Anhui Agricultural University, China (AAUF). Both illustration and comments accompany the description.

Key words—taxonomy, *Rhytismataceae*, *Oleaceae*

Introduction

Coccomyces De Not. is the second-largest genus in the *Rhytismataceae* (*Rhytismatales*, *Leotiomycetes*, *Ascomycota*) (Kirk et al. 2008). Members of this genus are characterized by polygonal to more or less circular ascomata opening by several radiate or irregular splits, cylindrical to clavate asci, and filiform to fusiform ascospores, often with gelatinous sheaths (Sherwood 1980; Cannon & Minter 1986; Johnston 1986, 2000; Spooner 1990; Lin et al. 1994).

Of the 116 *Coccomyces* species known worldwide (Kirk et al. 2008), 23 have been reported from China (Korf & Zhuang 1985; Lin 1998; Hou et al. 2006, 2007). They are widely distributed and inhabit leaves, twigs, bark, or wood of vascular plants, especially *Ericaceae*, *Fagaceae,* and *Lauraceae* (Sherwood 1980). Some species, such as *C. guizhouensis* Y.R. Lin & B.F. Hu, *C. ledi* Rehm, *C. leptideus* (Fr.) B. Erikss., *C. strobi* J. Reid & Cain, and *C. vilis* Syd. et al., may cause plant disease of economic significance (Lin et al. 1994, Rehm 1913, Sherwood 1980, Reid & Cain 1961, Cannon & Minter 1984).

In the present paper, *C. minimus* on leaves of *Osmanthus fragrans* (Thunb.) Lour. (*Oleaceae*) from Mt Huangshan in Anhui Province, China, is described as a new species.

Materials & methods

Pieces of plant material bearing fruit bodies containing asci and ascospores were selected. Sections of the fruitbodies 10–15 µm thick were cut using a freezing microtome and mounted in water, Melzer's reagent, 5% KOH, or 0.1% (w/v) cotton blue in lactic acid for microscopical observation. Gelatinous sheaths surrounding ascospores and paraphyses were observed in water or cotton blue in lactic acid. Measurements were made using material mounted in 5% KOH or Melzer's reagent from more than 20 ascospores, asci and paraphyses per ascoma. Point and line integrated illustrations of external shapes and internal structures of fruit bodies were prepared using a microscope drawing device.

Taxonomy

Coccomyces minimus Y.R. Lin, C.L. Hou & G.J. Jia, **sp. nov.** FIGS. 1–7

MycoBank 561773

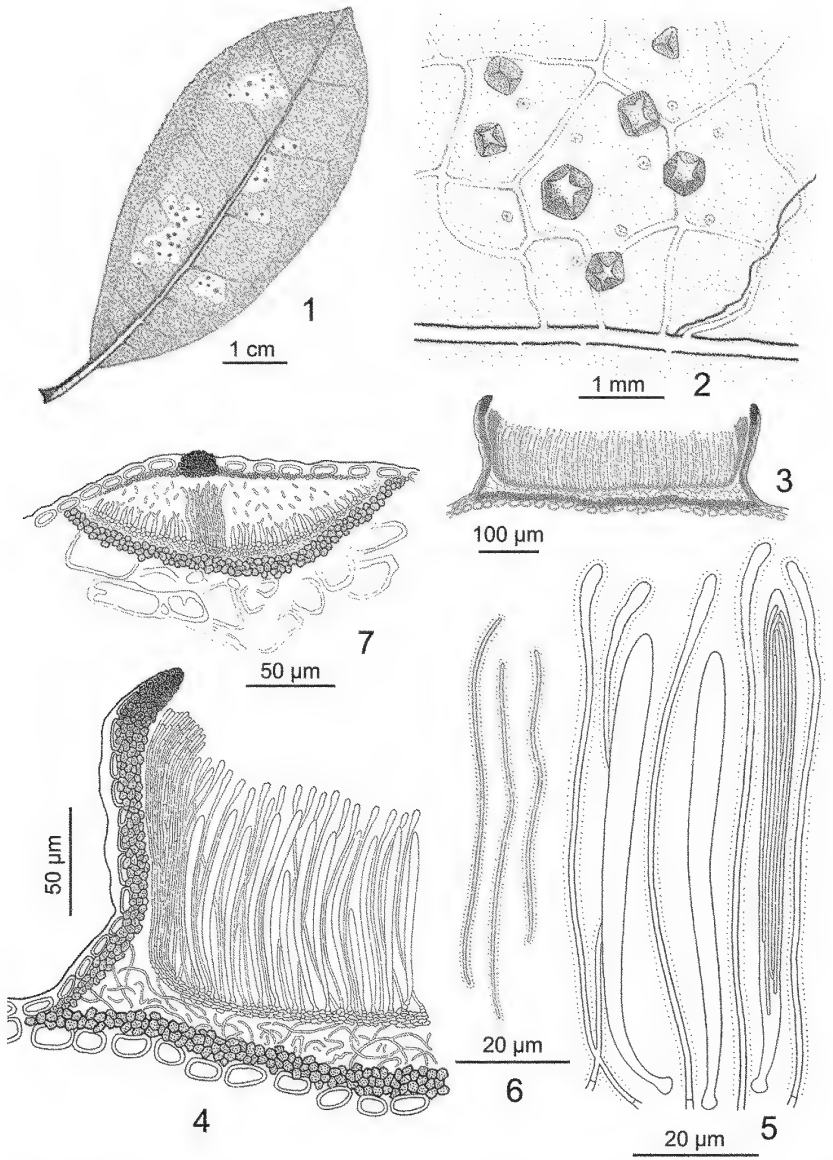
Ascomata amphigena, 300–690 µm, trigona usque ad hexagona, per lacinias 3–6 aperientia, subepidermalia. Cellulae labiorum et periphysoidei non visae. Excipulum ex hyphis septatis aliquantum parallelis constatum, apice 20–25 µm crassum. Paraphyses 85–95 × 1.0–1.5 µm, filiformes, ad apicem gradatim ad 2–3 µm incrassatae. Asci in successione maturescentes, 75–88 × 4–5 µm, clavati-cylindrici, brevi-stipitati, J–, 8-spori. Ascosporae 50–72 × 0.8–1.0 µm, filiformes, hyalinae, aseptatae, vagina gelatinosa ca 0.8 µm crassa indutae.

TYPE: China, Anhui, Mt Huangshan, Ciguangge, alt. 730 m, on *Osmanthus fragrans* leaves, 11 Sept. 2007, Y.R. Lin et al. 2227a (**Holotype** AAUF 68335a).

ETYMOLOGY: *minimus* (Latin = very little, smallest), referring to the size of the ascomata.

ZONE LINES frequent, grey-black or black, thin, entirely or partly surrounding the bleached spots.

CONIDIOMATA developing on both sides of leaves, mostly on the lower side of the leaf, scattered to crowded. In surface view, conidiomata 100–220 µm diam., rotund or slightly irregular, black-brown in the centre and the perimeter line of the conidioma, grey-brown elsewhere, somewhat raising the leaf surface, discharging spores through an apical ostiole. In vertical section, conidiomata subepidermal, more or less double lens-shaped. UPPER WALL poorly developed, dark brown, with indefinite structure. BASAL WALL 8–14 µm thick, black-brown, strongly black and brittle, consisting of 2–3(–4) layers of thick-walled angular cells 3–6 µm diam. SUBCONIDIOGENOUS LAYER 5–7 µm thick, composed of light brown, thin-walled angular cells. TRICHOGYNES 28–36 × 1.5–2 µm, subfiliform, septate. CONIDIOGENOUS CELLS 6.5–12 × 2–3



Figs. 1–7. *Coccomyces minimus* on *Osmanthus fragrans*. 1. A leaf bearing fruit bodies. 2. Ascomata and conidiomata observed under a dissecting microscope. 3. Ascoma in median vertical section. 4. Detail of ascoma in median vertical section. 5. Paraphyses and asci. 6 Discharged ascospores. 7. Conidioma in vertical section.

μm, flask-shape, holoblastic sympodially proliferating. Conidia 3–5 × ca 1 μm, cylindrical, hyaline, aseptate.

Ascomata in similar positions on the host, scattered to crowded in yellow-brown or grey-yellow, irregular bleached spots 5–12 mm diam. In surface view, ascomata black-brown to black, slightly shiny, triangular to hexagonal, 300–690 μm diam., strongly raising the substratum surface but depressed in the central area, with an obvious performed dehiscence mechanism, opening by 3–6 radial splits, which nearly extend to the edge of ascoma, to expose the waxy-yellow hymenium. Lips not seen. In median vertical section, ascomata subepidermal, 150–190 μm deep. Covering stroma 16–20 μm thick near the opening, slightly thinner towards the edge, connecting to the basal stroma, consisting of textura angularis-globulosa with dark brown or black-brown, thick-walled cells 3–5 μm diam. Periphysoids not seen. Basal stroma 12–22 μm thick, strongly black and brittle, composed of textura angularis with 2–3(–4) layers of black-brown, thick-walled cells 4–6 μm diam. Internal matrix of stroma well developed, 12–25 μm thick, consisting of hyaline, gelatinised textura intricata. Excipulum arising from the marginal paraphyses, 20–25 μm wide above, becoming thinner towards the base, comprised of rows of hyaline, multi-septate hyphae. Subhymenium 10–15 μm thick, comprised of hyaline textura angularis-porrecta. Paraphyses 85–95 × 1.0–1.5 μm, filiform, not branched, often gradually swollen to 2–3 μm above, covered with a ca 1 μm thick gelatinous matrix but not forming an epithecium. Asci ripening sequentially, 75–88 × 4–5 μm, cylindrical-clavate, short-stalked, thin-walled, apex round, J–, 8-spored. Ascospores fasciculate, 50–72 × 0.8–1.0 μm, hyaline, filiform, aseptate, slightly tapered towards the base, covered by a ca 0.8 μm thick gelatinous sheath.

Host species, habitat and distribution: *Osmanthus fragrans*; producing conidiomata and ascomata on fallen leaves. Known only from the type locality, Anhui, China.

Comments—*Coccomyces minimus* resembles *C. cyclobalanopsis* Y.R. Lin & Z.Z. Li in the shape of paraphyses, asci, and ascospores as well as in the color of the exposed hymenium. However, *C. cyclobalanopsis* has larger, intraepidermal ascomata (400–900 μm diam.) without conidiomata, a thinner basal stroma (7–10 μm thick), a well-developed subhymenium (20–28 μm thick), much longer paraphyses (110–130 μm) and asci (90–115 μm), and an upper part of the excipulum 30–50 μm thick arising from inner cells of the covering stroma (Lin et al. 2000).

Coccomyces multangularis Y.R. Lin & Z.Z. Li is easily distinguished from *C. minimus* by its 3–9-sided much larger (500–1100 μm diam.) ascomata, the 35–50 μm thick upper portion of the excipulum, infrequent periphysoids, and slightly longer asci (85–105 μm) (Lin et al. 2001).

The similar *C. concolor* Sherwood differs in its orbicular ascomata lacking a performed dehiscence mechanism, the covering stroma comprising 1–2 layers of loosely interwoven brown hyphae 2–3 µm diam. that are not black and brittle, the thicker (40–50 µm) excipulum, the swollen (5–6 µm) paraphyses apices, and the much longer asci (80–120 µm) and ascospores (70–100 µm) (Sherwood 1980).

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New records of poaceous rusts from Pakistan

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Abstract — *Puccinia stipae* var. *stipae* and *Uromyces sporobolicola* are described and illustrated as new records for Pakistan. *Calamagrostis pseudophragmites* for *Puccinia sessilis*, *Agrostis gigantea* for *P. recondita*, and *Dactylis glomerata* for *P. striiformis* are new poaceous hosts for these rust fungi from Pakistan.

Key words — graminicolous, *Uredinales*, weeds

Introduction

Poaceous weeds are an important competitor of all crop plants worldwide as well as in Pakistan. Economically important grass species are threatened by invasive weeds, and several host-specific rust fungi have been used as biological control agents against certain noxious weeds (Yandoc-Ables et al. 2006). There are about 158 genera (492 species) of plants from the family *Poaceae* in Pakistan. Among the 100 rust species known to infect 130 plant species (Afshan et al. 2010) are 70 species of *Puccinia* Pers. and 14 species of *Uromyces* (Link) Unger (Afshan & Khalid 2009; Afshan et al. 2009, 2010a,b, 2011; Iqbal et al. 2009; Khalid & Afshan 2009).

In this paper we report *Puccinia stipae* var. *stipae* on *Stipa* sp. and *Uromyces sporobolicola* on *Sporobolus marginatus* as new records and identify new poaceous hosts for *Puccinia sessilis* (*Calamagrostis pseudophragmite*), *P. recondita* (*Agrostis gigantea*), and *P. striiformis* (*Dactylis glomerata*) in Pakistan.

Materials & methods

Infected plants with their inflorescences were collected from sampling sites throughout Pakistan. The rusted specimens were photographed and free hand sections and scrape mounts of infected portions were observed under the stereomicroscope. Twenty spores of each spore state found were examined under microscope (Nikon YS 100), and spore dimensions were determined using a Zeiss eyepiece screw micrometer.

Sections and spores were photographed by digiporo-Labomed and illustrated using a Leitz camera lucida (Wetzlar Germany).

Enumeration of taxa

Uromyces sporobolicola J.C. Lindq., Revta Fac. Agron. Vet. Univ. nac. La Plata, Ser. 3, 38: 89 (1963 ["1962"]). FIG. 1

SPERMOGONIA and AECIA not found. UREDINIA adaxial, cinnamon-brown; UREDINIOSPORES light brown to hyaline, echinulate, globose to subglobose, (19.5–)25–33 × (22–)2–38 µm; wall 1.6–2.6 µm thick, germ pores 2–4, supraequatorial. TELIA amphigenous, blackish, covered by the epidermis; TELIOSPORES 1-celled, brown, ovate, 19–21.5 × 21.5–27 µm; apex 2.4–5.4 µm thick, wall 1.4–2 µm thick; pedicel short, ≤ 11 µm long.

MATERIAL EXAMINED: **PAKISTAN**, **PUNJAB**, Sialkot, at 234 m a.s.l., on leaves of *Sporobolus marginatus* Hochst. ex A. Rich., stages II + III, 20 April 2011, AIM # 27 (LAH Herbarium No.1130).

COMMENTS: Cummins (1971) reported *U. sporobolicola,* here a new record for Pakistan, on *Sporobolus pyramidatus*. Other rusts known to infect *Sporobolus*

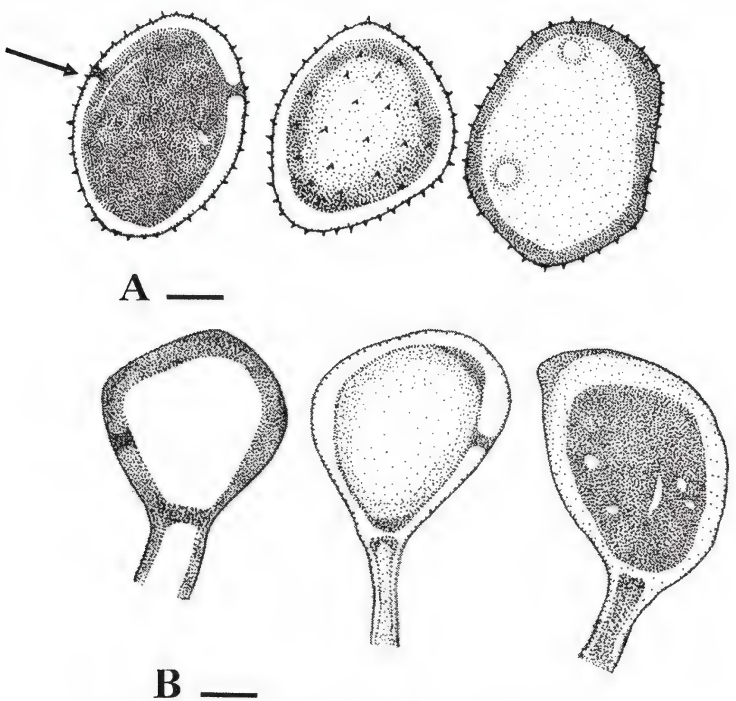

FIG. 1: *Uromyces sporobolicola* (lucida drawing; scale = 10 µm).
A. Urediniospores showing germ pores (arrowed); B. One-celled teliospores.

species (*S. arabicus, S. coromandelianus*) in Pakistan include *Puccinia sporoboli-arabici* Afshan et al., *P. sporoboli-coromandeliani* Afshan et al., *Uromyces ignobilis* (Syd. & P. Syd.) Arthur and *U. tenuicutis* McAlpine from Lahore (Ahmad 1956a,b, 1976, Masood et al. 1995) and NWFP (Afshan et al. 2008, 2010a).

Puccinia stipae (Opiz) Arthur, Bull. Iowa Agric. Coll. Dept. Bot.: 160 (1884)
var. *stipae* FIG. 2

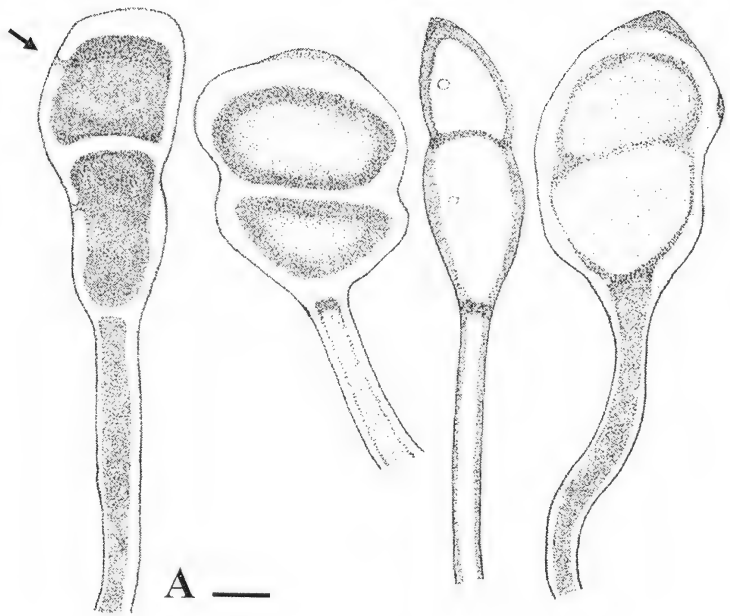

FIG. 2: *Puccinia stipae* var. *stipae* (lucida drawing; scale = 13 μm). Teliospores showing germ pores (arrows).

SPERMOGONIA, AECIA, & UREDINIA not found. TELIA blackish brown, adaxial, exposed, compact, 0.14–0.27 × 0.13–0.19 mm. TELIOSPORES golden brown, broadly ellipsoid, 23–34 × 45–63 μm; wall 1.9–2.6 μm thick at the sides, 4.5–8 μm thick apically; pedicel long, broken mostly, hyaline, 5.5-9 μm wide, ≤ 112 μm long.

MATERIAL EXAMINED: **PAKISTAN**, **GILGIT & BALTISTAN**, Skardu, Shigar valley, at 3000 m a.s.l., on leaves of *Stipa* sp., stage III, 20 July 2010, AIM # 38 (LAH Herbarium No.1131).

COMMENTS: (Cummins (1971) previously reported *P. stipae* var. *stipae*, here a new record for Pakistan, on *Stipa spartea*. Ahmad (1969) reported a different variety, *Puccinia stipae* var. *stipina* (Tranzschel) H.C. Greene & Cummins (≡ *Puccinia stipina* Tranzschel), on *Stipa himalaica* from Quetta in Pakistan.

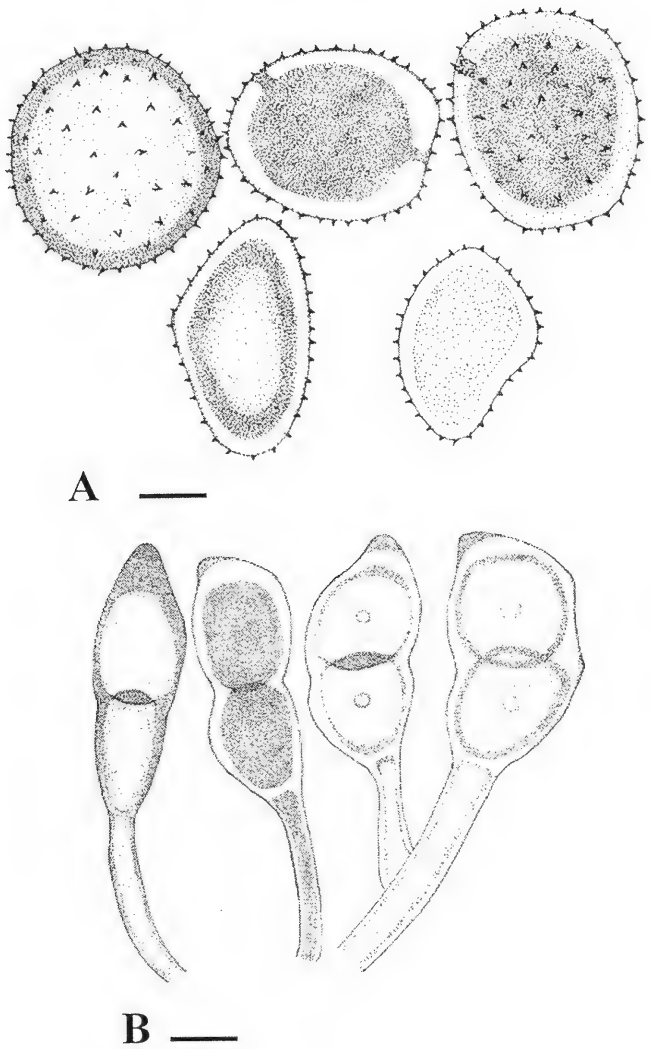

Fig. 3: *Puccinia sessilis* var. *sessilis* (lucida drawings; scale = 14 µm).
A. Urediniospores showing echinulate surface ornamentation and germ pores; B. Teliospores.

Puccinia sessilis W.G. Schneid., Jahresber. Schles. Ges. Vaterl. Cult. 49: 19 (1871)
var. *sessilis* Fig. 3

Spermogonia and aecia not found. Uredinia on stipe, brown, naked. Urediniospore light brown to hyaline, globose to subglobose, 21–32 × 24–36 µm; wall 1.9–2.4 µm; germ pores up to 4, scattered or tending to be equatorial.

Telia amphigenous, blackish, covered by epidermis. Teliospore chest nut brown, 23–32 × 47–68 µm; wall 1.5–3 µm thick at sides, 6.5–13 µm thick apically, pedicel ≤ 77 µm long.

Material examined: **PAKISTAN, Gilgit & Baltistan,** Skardu, Shigar valley, at 3000 m a.s.l., on leaves of *Calamagrostis pseudophragmites* (Haller f.) Koeler, stages II + III, 21 July 2010, AIM # 39 (LAH Herbarium No.1132).

Comments: Khalid & Iqbal (1996) previously reported *P. sessilis* on leaves of *Phalaris minor* from Deosai Plains near Pakora Lake. *Calamagrostis pseudophragmites* is a new host for the variety in Pakistan.

Puccinia striiformis Westend., Bull. Acad. Roy. Sci. Belgique 21: 235 (1854)
var. *striiformis* Fig. 4

Spermogonia and aecia not found. Uredinia light brown, adaxial, in linear series. Urediniospores globose to subglobose, hyaline, echinulate, 24–31 × 27–36 µm; Wall 1.4–2.8 µm thick, germ pores 2–many, scattered. Paraphyses

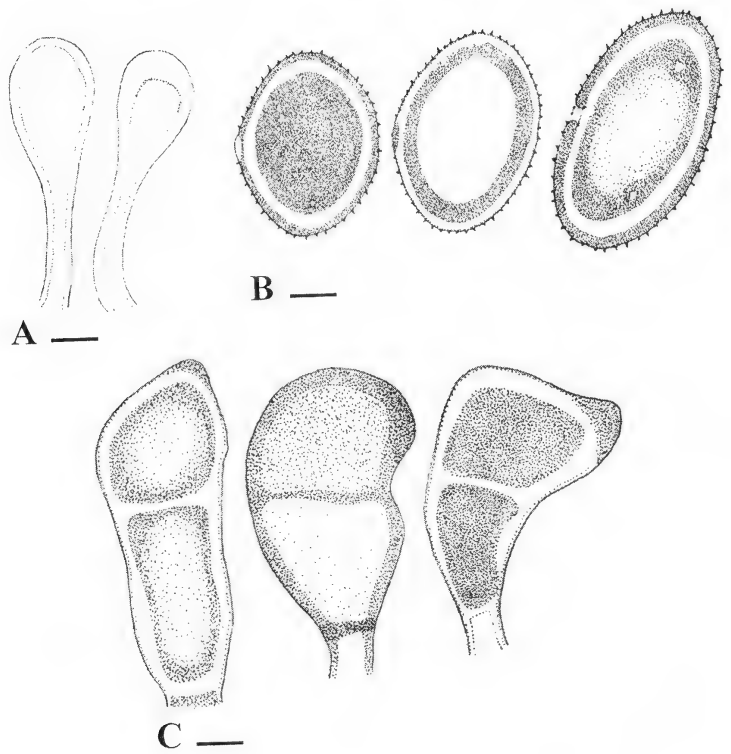

Fig. 4: *Puccinia striiformis* var. *striiformis* (lucida drawings; scale = 13 µm).
A. Paraphyses; B. Urediniospores showing germ pores (arrowed); C. Teliospores.

capitate, having cap of 6.5–8.0 µm diameter. Telia blackish, abaxial, sheaths in linear series, covered by epidermis, with hyaline paraphyses, forming locules. Teliospores clavate, light brown, 2-celled, 13–19(–22.0) × 34.0–50.0 µm; sometimes upper cell thicker than lower, apex 3.0–7.0(–9.0) µm thick, wall 1–2 µm thick, pedicel very delicate.

Material examined: **PAKISTAN**, **Gilgit & Baltistan**, Fairy Meadows, at 3036 m a.s.l., on *Dactylis glomerata* L., stages II + III, 12 August 2007, AIM # 20 (LAH Herbarium No.1133).

Comments: Numerous authors have reported *P. striiformis* var. *striiformis* on *Triticum aestivum*, *Hordeum vulgare*, and *Poa* sp. from Quetta, Kalat, Tandojam, Rawalpindi, Nathia Gali, Shogran (Kaghan valley), and Lahore (Ahmad 1956a,b, Khan & Kamal 1968, Malik et al. 1968, Malik & Virk 1968, Kakishima et al. 1993) and on *Leersia oryzoides* from Fairy Meadows (Afshan 2009). *Dactylis glomerata* is a new host for the variety in Pakistan.

Puccinia recondita Desm., Bull. Soc. bot. Fr. 4: 798 (1857) Fig. 5

Spermogonia and aecia not found. Uredinia abaxial, brown. Urediniospores globose to subglobose, hyaline, 15–21 × 17–26 µm; walls 1.4–2.6 µm thick, echinulate; germ pores 2–many, scattered. Telia abaxial, black, covered by epidermis, 0.1–0.2 × 0.04–0.05 mm. Teliospores light

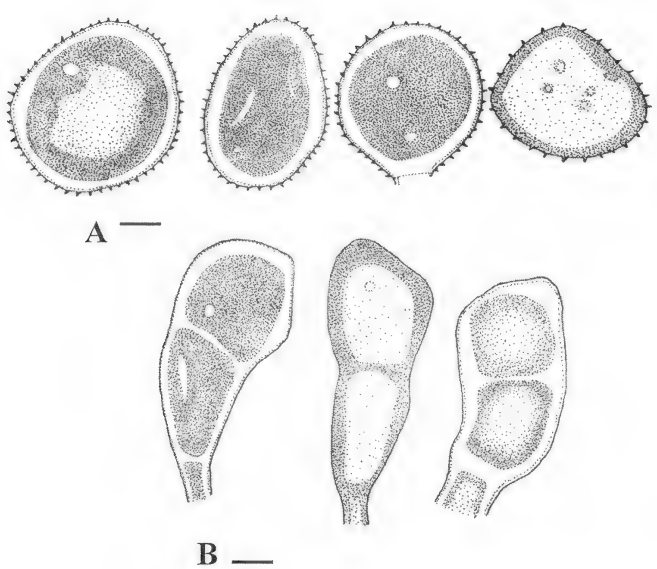

Fig. 5: *Puccinia recondita* (lucida drawings; scale = 13 µm).
A. Urediniospores showing germ pores; B. Teliospores.

brown to hyaline, clavate, 2-celled, 13–19(–21) × 38–52 µm; wall 1.6–2.4 µm thick, apex 3–6 µm thick; pedicel very delicate.

Material examined: **PAKISTAN**, **Khyber-Pakhtunkhawah** (KPK), Sharan, at 2752 m a.s.l., on *Agrostis gigantea* Roth, stages II + III, 27 July 2007, AIM # 19 (LAH Herbarium No.1134).

Comments: *Agrostis gigantea* is a new host from Pakistan for *Puccinia recondita,* which has previously been reported on *Agropyron* sp., *Aquilegia fragrans*, *A. pubiflora*, *Brachypodium* sp., *Eremopyrum bonaepartis*, *Lolium perenne*, *Triticum aestivum*, and *Thalictrum minus* from Swat, Kaghan valley, Ayubia, Tandojam, Shogran to Sari, Sharan, Murree, Faisalabad, and Quetta (Ahmad 1956a, Hasnain et al. 1959, Jørstad & Iqbal 1967, Ghaffar & Kafi 1968, Khan & Kamal 1968, Malik & Virk 1968, Malik et al. 1968, Ahmad & Arshad 1972, Ono 1992, Ono & Kakishima 1992).

Acknowledgements

We sincerely thank Dr. Abdul Rehman Niazi (Department of Botany, University of the Punjab, Lahore, Pakistan) and Dr. Omar Paíno Perdomo (Dominican Society of Mycology, Santo Domingo, Dominican Republic) for their valuable suggestions to improve the manuscript and acting as presubmission reviewers. We are also thankful to Dr. Shaun Pennycook, Nomenclature Editor, for reviewing the manuscript critically.

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New records of smut fungi. 5

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Abstract — Morphological study of an anthericolous smut fungus on *Scilla sardensis* (*Chionodoxa sardensis*) and *S. luciliae* (*Chionodoxa luciliae*) from UK has shown it to represent *Antherospora scillae.* This is also known in UK on *Scilla verna* and *S. forbesii,* new host records for this species. The new species, *Urocystis bolboschoeni* on *Bolboschoenus maritimus,* is described and illustrated from UK.

Key words — taxonomy, *Urocystidales, Ustilaginomycetes*

Introduction

In the current taxonomic scheme of the *Ustilaginomycetes,* the genus *Ustilago* is restricted to species parasitizing plants of *Poaceae* (Vánky 1999, 2002). For species on *Liliaceae* s. lat. previously referred to *Ustilago,* Ershad (2000) erected a new genus, *Vankya,* with three species: *V. heufleri* (Fuckel) Ershad on *Erythronium* and *Tulipa; V. ornithogali* (J.C. Schmidt & Kunze) Ershad on *Gagea;* and *V. vaillantii* (Tul. & C. Tul.) Ershad on *Albuca, Bellevalia, Eucomis, Hyacinthus, Muscari, Puschkinia, Scilla* (including *Chionodoxa*), and *Urginea. Vankya* was later restricted to the species on leaves and stems of liliaceous plants: *V. heufleri, V. ornithogali,* and the recently described *V. lloydiae* Vánky on *Lloydia triflora* (Ledeb.) Baker (Vánky 2009a). For the anthericolous smut fungus *Vankya vaillantii* s. lat. on plants of *Asparagaceae* (syn. *Hyacinthaceae; Liliaceae* p.p.), Bauer et al. (2008) described a new genus, *Antherospora,* with seven species: *A. albucae* (Syd. & P. Syd.) R. Bauer et al. on *Albuca, A. peglerae* (Bubák et al.) R. Bauer et al. on *Ornithogalum, A. scillae* on *Scilla bifolia* L., *A. tourneuxii* (A.A. Fisch. Waldh.) R. Bauer et al. on *Bellevalia, A. urgineae* (Maire) R. Bauer et al. on *Urginea, A. vaillantii* (Tul. & C. Tul.) R. Bauer et al.

on *Muscari,* and the cryptic species *A. vindobonensis* R. Bauer et al. on *Scilla vindobonensis* Speta. An eighth species, *A. eucomis* Vánky on *Eucomis,* was added by Vánky (2009b). The narrow host association of these anthericolous smut fungi (Bauer et al. 2008, Vánky 2009b) warranted closer study of the morphology of *Antherospora* species on *Chionodoxa* (currently treated as members of *Scilla*). The result of this study is presented here.

In June 2010, two specimens of *Urocystis* in leaves of *Bolboschoenus maritimus* were collected by Mr N.W. Legon in South Devon, England. No *Urocystis* has been previously recorded on *Bolboschoenus* nor on plants of related genera *(Scirpus, Holoschoenus, Isolepis, Schoenoplectus, Trichophorum).* We consider those specimens as belonging to a new species, described below.

Material & methods

Dried specimens from the mycological collection of the Royal Botanic Gardens, Kew [K(M)] were examined under light (LM) and scanning electron (SEM) microscopes. For LM observations, spores were mounted in lactophenol solution on glass slides, gently heated to boiling point to rehydrate the spores, and then cooled. Spore measurements are given in the form: min–max [mean ± 1 standard deviation]. In the description, the total number of spores (n) from all collections (x) measured are given in the form '(n/x)'. For SEM, spores were attached to specimen holders by double-sided adhesive tape and coated with gold with an ion sputter. The surface structure of spores was observed at 10 kV and photographed with a JEOL JSM-5510 scanning electron microscope.

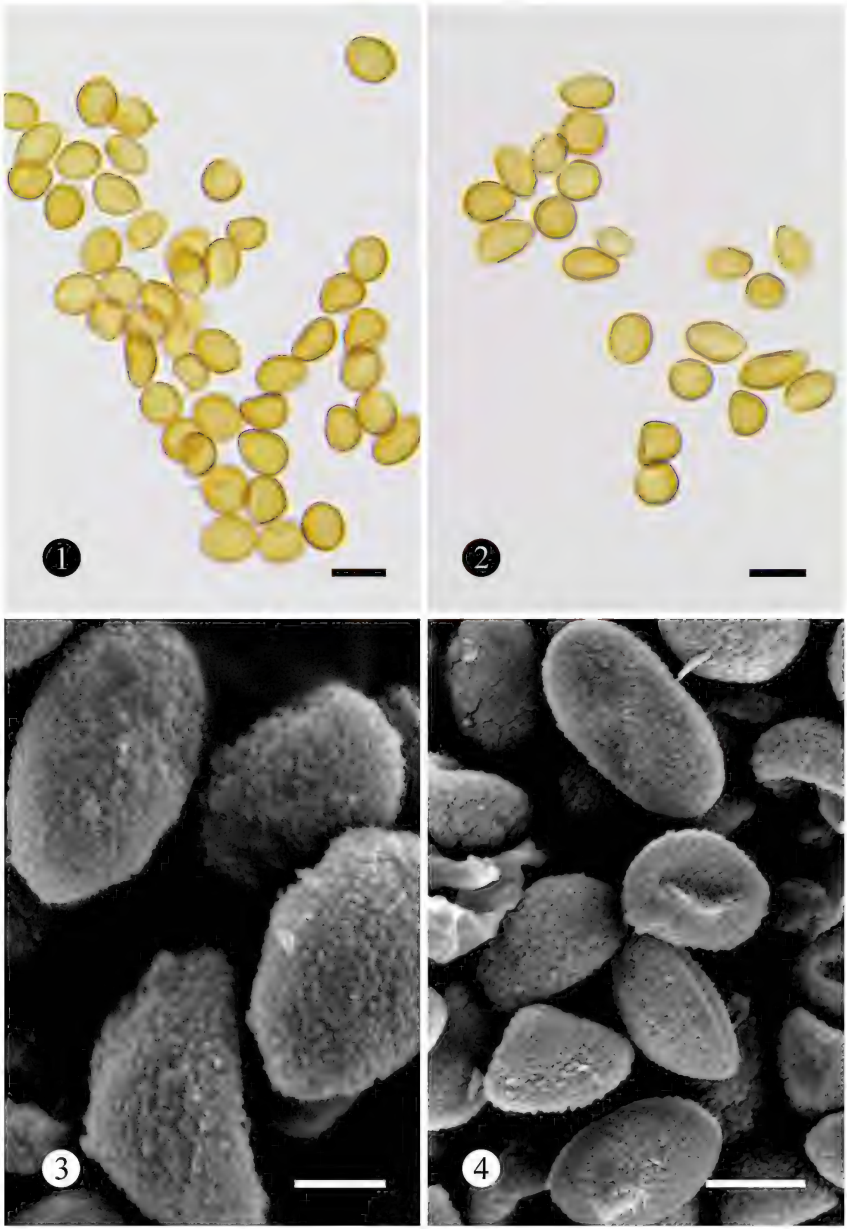
Taxonomy

Antherospora scillae (Cif.) R. Bauer et al., Mycol. Res. 112: 1301, 2008. Figs 1–4

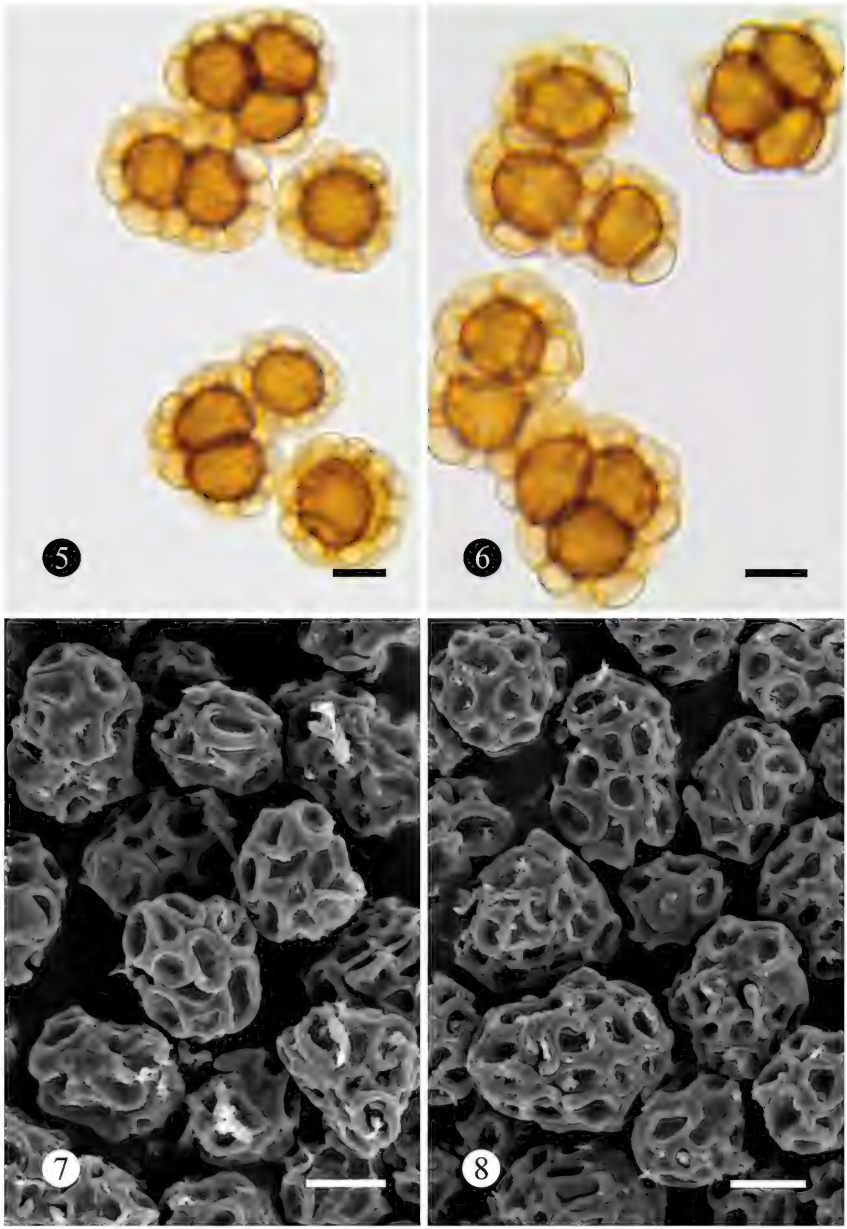
Sori in the anthers and on the surface of anther filaments. Spore mass powdery, dark olivaceous brown. Infection systemic, all anthers are infected. Spores variable in shape, globose, subglobose, broadly ellipsoidal, ovoid, elongated or slightly irregular, sometimes pyriform, 7–12.5 × 6.5–9.5 [9.4 ± 1.2 × 7.8 ± 0.8] µm ($n/_2$ = 200), light olivaceous brown; wall even, 0.7–1.0 µm thick, densely verruculose.

Specimens examined — On ***Scilla sardensis*** (Whittall ex Barr & Sugden) Speta: **UK, ENGLAND, Surrey,** Kew, Royal Botanic Gardens, 21 April 1947, leg. G.M. Waterhouse [ex herb. University of Sheffield, no. 764; IMI 15342] (as *Ustilago vaillantii* on *Chionodoxa sardensis* Whittall ex Barr & Sugden, K(M) 116304). — On ***Scilla luciliae*** (Boiss.) Speta: **UK, ENGLAND, Surrey,** Kew, Royal Botanic Gardens, near Lion Gate, 23 March 1954, leg. R.W.G. Dennis (as *Ustilago vaillantii* on *Chionodoxa luciliae* Boiss., K(M) 69569).

Comments — The study of this fungus on these two hosts was provoked by the high host specialization amongst *Antherospora* species infecting *Scilla* with the presumption that *A. scillae* is restricted to *Scilla bifolia* as suggested by Bauer et al. (2008: 1302). Bauer et al. (2008) described both *Antherospora scillae* on *Scilla bifolia* and *A. vindobonensis* on *Scilla vindobonensis* as having



Figs 1–4. Spores of *Antherospora scillae*. 1–2 on *Scilla luciliae* (K(M) 69569) in LM, 3 on *S. sardensis* (K(M) 116304) in SEM, 4 on *S. luciliae* (K(M) 69569) in SEM. Scale bars: 1–2 = 10 µm, 3 = 2 µm; 4 = 5 µm.



Figs 5–8. Spores of *Urocystis bolboschoeni* on *Bolboschoenus maritimus*.
1–2 in LM (holotype), 3 in SEM (K(M) 166454), 4 in SEM (holotype).
Scale bars: 5–8 = 10 μm.

spore walls with a thickness of ca 0.5 µm. The specimens we examined on *Scilla sardensis* and *S. luciliae* have spores with a thicker wall (0.7–1.0 µm wide), and it seemed warranted to make additional observations of the spore wall on *S. bifolia*. We found that the spore wall thickness of two specimens on *S. bifolia* measured 0.7–0.9 µm (Vánky *Ustilag.* exs. no. 57) and 0.8–1.1 µm (SOMF 19206). Additionally, Vánky (2009b) cited a spore wall thickness of 0.8–1.5 µm for *Antherospora scillae*. Our spore measurements from four specimens of *A. scillae* on *Scilla bifolia* (Vánky *Ustilag.* exs. no. 57; SOMF 2859, 2888, & 19206) (7–14.5 × 6.5–10 [9.8 ± 1.1 × 8.5 ± 0.7] µm; $n/_4 = 350$) resembled those of the British specimens we examined.

Additional specimens examined — On ***Scilla bifolia:*** **HUNGARY,** prope pag. Makád insulae Csepel sziget, ca. 98 m, 6 April 1965, S. Tóth (Vánky *Ustilag.* exs., no. 57); **BULGARIA,** l.d. Papazova Korija prope urb. Elhovo, 21 March 1962, C. Hinkova (SOMF 2859); l.d. Gorna Topčija, distr, Jambol, 22 March 1961, C. Hinkova SOMF 2888); Pirin Mts, Sinanitsa, ca. 2000 m, 24 May 1985, C.M. Denchev (SOMF 19206).

Currently, there is no molecular phylogenetic inference for distinguishing the anthericolous smut fungus on *Scilla sardensis* and *S. luciliae* from that on *S. bifolia*. Because no morphological differences were found, we identified *Antherospora scillae* as the fungus on the British specimens that we examined.

This anthericolous smut fungus known in UK on *Scilla verna* Huds. (several collections) has been recently collected also on *S. forbesii* (Baker) Speta (≡ *Chionodoxa forbesii* Baker) [K(M) 169982]. *Scilla sardensis, S. luciliae, S. verna,* and *S. forbesii* are new host records for *Antherospora scillae.*

Urocystis bolboschoeni Denchev, T. Denchev, Spooner & Legon, **sp. nov.** Figs 5–8

MycoBank MB 561704

Sori in foliis et vaginis foliorum strias formati. Glomeruli sporarum 16–40 × 14.5–28.5 µm, e sporis 1–3 (–5) compositi [numeri sporarum: 1 = 44.6%, 2 = 39.2%, 3 = 11.2%, 4 = 4.2%, 5 = 0.8%], strato cellularum sterilium perfecte circumdatis composite. Sporae 12.5–18.5 × 10–15 [15.2 ± 1.1 × 12.7 ± 1.1] µm, rufobrunneae, pariete 0.6–0.9 µm crasso.

Type on *Bolboschoenus maritimus* (L.) Palla: UK, England, South Devon, Budleigh Salterton, Otter Estuary, 29 June 2010, leg. N.W. Legon, as *Urocystis* sp. (**holotype,** K(M) 166455).

Etymology: the name refers to the host genus.

Sori in leaves and leaf sheaths between the veins, as streaks, sometimes confluent, initially covered by the epidermis, rupturing longitudinally to expose a powdery, dark reddish brown mass of spore balls. Spore balls subglobose to ellipsoidal or slightly irregular, composed of 1–3 (–5) central teliospores [1 = 44.6%, 2 = 39.2%, 3 = 11.2%, 4 = 4.2%, 5 = 0.8%; $n/_2 = 950$] and a continuous layer of peripheral sterile cells; 16–25 × 14.5–22.5 µm [with 1 teliospore], 21–33.5 × 15.5–24.5 µm [with 2 teliospores], 25–40 × 17–28.5 µm [with 3 teliospores]. Sterile cells broadly elliptical, elliptical, suborbicular or ovate

in outline, or collapsed, sometimes slightly irregular, 5–11.5 µm long, pale or yellowish, wall 0.6–1.0 µm thick, smooth. Spores globose, subglobose, broadly ellipsoidal or ovoid, 12.5–18.5 × 10–15 [15.2 ± 1.1 × 12.7 ± 1.1] µm ($n/_2$ = 200), medium reddish brown; wall 0.6–0.9 µm thick, finely verruculose.

Distribution — On *Cyperaceae: Bolboschoenus maritimus,* Europe (England). Known only from the type locality.

Additional specimens examined — On ***Bolboschoenus maritimus:*** **UK, England, South Devon,** Budleigh Salterton, Otter Estuary, 22 June 2010, leg. N.W. Legon (as *Urocystis* sp., K(M) 166454); 12 May 2011, leg. N.W. Legon (K(M) 170764).

Comments — On *Cyperaceae* three *Urocystis* species have been described: (i) *U. fischeri* Körn. ex G. Winter on species of *Carex,* with type on *C. acuta* L. from Germany; distributed in Europe, Asia, and North America (sori in leaves and culms, spore balls 20–40 µm long, composed of 1–3 (–4) fertile spores); (ii) *U. littoralis* (Lagerh.) Zundel on *Carex maritima* Gunnerus from Norway (sori in leaves, spore balls 25–50 µm long, composed of (1–) 2–6 (–9) fertile spores); and (iii) *U. chorizandrae* Cunningt. et al. on *Chorizandra enodis* Nees from Australia (sori in leaves and sterile culms, spore balls 25–50 µm long, composed of 1–5 (–8) fertile spores) (Vánky & Shivas 2003, Vánky 2009c).

It is remarkable that no smut has been previously reported on this common and widely distributed host species.

Acknowledgements

We gratefully acknowledge Dr Kálmán Vánky (Herbarium *Ustilaginales* Vánky, Tübingen, Germany) and Dr Roger G. Shivas (Agri-Science Queensland, Australia) for critically reading the manuscript and serving as pre-submission reviewers.

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A new species of *Marasmius* from northern Argentina

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Abstract — *Marasmius tyrius* is proposed as a new species. It is characterized by collariate lamellae, insititious stipe, violet pileus, pileipellis with Siccus-type broom cells, and clavate to fusoid spores. Detailed description and illustrations of macro- and microscopic characters are provided.

Key words — Argentinean mycobiota, *Marasmiaceae*, taxonomy

Introduction

The genus *Marasmius* (*Marasmiaceae, Agaricales*) is represented by a large number of species, especially in the tropics (Singer 1976, 1986). It is characterized by a hymeniform pileipellis composed of broom cells of Siccus- or Rotalis-type, or of exclusively smooth cells. Siccus-type cells are characterized by apical appendages or more or less erect setulae, while the Rotalis-type usually have short appendages and that descend from the apex down towards the middle of the cell (Singer 1986).

Spegazzini (1880a,b, 1883, 1887, 1891, 1898, 1902, 1909, 1925, 1926a,b), Singer (1950, 1959, 1965, 1969, 1976, 1989), and Singer & Digilio (1953) described species of *Marasmius* from Argentina, Chile, and Paraguay. Singer (1953) later studied several types of species described by Spegazzini and included his observations in the exhaustive monographs on *Marasmius* in South America and Neotropics (Singer 1959, 1965, 1976). After these reports, mycological literature on the genus in the region has been scant. Recently, there has been a renewed worldwide interest in *Marasmius* (Desjardin et al. 2000, Antonín 2003, 2004a,b, 2007, Wannathes et al. 2004, 2009, Antonín & Buyck 2006, Desjardin & Ovrebo 2006, Puccinelli & Capelari 2006, Tan et al. 2009, Antonín & Noordeloos 2010). In Argentina, Lechner et al. (2006) reported new records of *Marasmius* collected in the northern region, and several species have

been described and illustrated in the Pictorial Atlas of Iguazú National Park (Wright et al. 2008).

According to our knowledge, one specimen collected during an expedition in northern Argentina does not match any described *Marasmius*. Thus, we propose *Marasmius tyrius* as a new species.

Materials & methods

The specimen was photographed and its macroscopic features were recorded when fresh. Tissues were mounted in 5% KOH + 1% aqueous phloxine or Melzer's reagent and observed under a Nikon E-600 microscope. For basidiospore measurements, the length : width quotient (Q) was calculated, with Qm indicating the mean value of Q quotients. Line drawings were made with the aid of a light tube. Basidiomata were dried, kept frozen for a week, and deposited in the BAFC herbarium (Department of Biodiversity and Experimental Biology, Faculty of Exact and Natural Sciences, Universidad de Buenos Aires).

Taxonomy

Marasmius tyrius B.E. Lechner & Papinutti, **sp. nov.** FIGS. 1–2

MycoBank MB 518617

Pileo 4–5.5 mm lato, 2–2.7 mm alto, hemisphaerico, centro depresso, violaceo, glabro, sulcato. Lamellis albis, subdistantibus (proxime 17), collariatis. Stipite castaneo, glabro, 31–37 × 0.2–0.5 mm. Basidiosporis 11.1–14.1 × 2.5–3.7 µm, clavatis ad fusiformibus, laevibus, hyalinis, inamyloideis. Basidiis clavatis, 23–28 × 4–6 µm, 1–3 sterigmata. Cheilocystidiis difformibus: (1) elementis typi Marasmii sicci, *17.3–20.5 × 3.7–9.5 µm, setulis 2–4 µm longis, tenuitunicatis vel crassitunicatis, castaneis in KOH, et (2) clavatis, 22.5–27.4 µm longis, tenuitunicatis, hyalinis. Elementis epicuticularibus plerumque typi M*arasmii sicci, *11.9–22.5 × 11.2–13.1 µm, setulis 4–5 µm longis.*

TYPE: Argentina, Chaco, Colonia Benítez, 27°26′19.20″S 58°50′51.39″W, on fallen leaves, 19-IX-2007, col. L. Papinutti, G. Rolón (**HOLOTYPE** BAFC 51726).

ETYMOLOGY: The epithet refers to the similarity of the pileus color to Tyrian purple, the dye famously produced in the ancient Phoenician city of Tyre (Latin: *Tyrus*).

PILEUS 4–5.5 mm in diam × 2–2.7 mm high, hemispheric, depressed at the centre, glabrous, deeply sulcate, violet (Plate 42, K10K11, Maerz & Paul 1930), centre light brown with an outer light violet ring. PILEUS CONTEXT white, very thin (less than 1 mm). LAMELLAE (Fig. 1c) collariate, subdistant (ca. 17), whitish, entire, lamellulae absent; edge broad, concolorous with the pileus. STIPE 31–37 × 0.2–0.5 mm, chestnut-brown, creamy to whitish at the apex, glabrous, insititious, cylindrical. RHIZOMORPHS frequently present, thin, black. STIPE CONTEXT thin, white.

BASIDIOSPORES (9.3–)11.1–14.1(–15.4) × 2.5–3.7 µm, Q = 3.24–4.97, Qm = 4.23, n = 30, clavate to fusoid, hyaline, inamyloid, smooth, thin-walled. BASIDIA clavate, 23–28 × 4–6 µm, 1–3 spored. Basidioles clavate, 19.2–25.0 µm long. PLEUROCYSTIDIA absent. LAMELLA EDGE sterile, with crowded cheilocystidia.

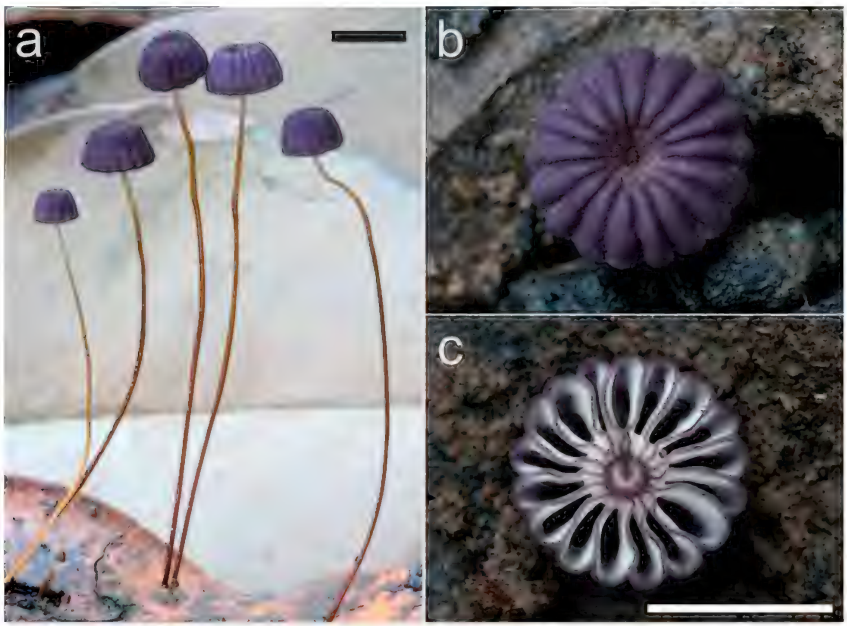

Fig. 1. Basidiomes of *Marasmius tyrius*.
Scale bar = 1 cm.

Cheilocystidia of two types: (1) Siccus-type broom cells, body 17.3–20.5 × 3.7–9.5 µm, setulae 2–4 µm long, thin- to thick-walled, castaneous in KOH, and (2) clavate, 22.5–27.4 µm long, thin-walled, hyaline. Hymenophoral trama regular to subregular; hyphae hyaline, clamped, 2.1–6.0 µm diam. Pileipellis composed of Siccus-type broom cells, body 11.9–22.5 × 11.2–13.1 µm, setulae 4–5 µm long, thin- to thick-walled, castaneous in KOH, mostly subvesiculose; and of clavate, some bifurcate, 20.1–34.1 × 5.4–7.0 µm, thin- to thick-walled elements (wall up to 2.3 µm thick), castaneous in KOH. Hyphae of pileus context hyaline, thin-walled, clamped, 2.6–6.0 µm diam, weakly dextrinoid. Hyphae of stipe context generative, hyaline, clamped, thin-walled, 2.6–5.2 µm diam, and thick-walled (up to 1.5 µm), 4.2–6.3 µm diam, scant in upper zones of the apex; non-dextrinoid.

Ecology and distribution — Gregarious, abundant in wet forest soil and litter at Colonia Benítez. Growing on fallen leaves of *Phoebe porphyria* (Griseb.) Mez (*Lauraceae*). Known only from type locality.

Comments — *Marasmius tyrius* belongs to sect. *Marasmius,* subsect. *Sicciformes* Antonín based on the presence of a collarium, the insititious stipe, and Siccus-type broom cells in the pileipellis. In his monograph on Neotropical

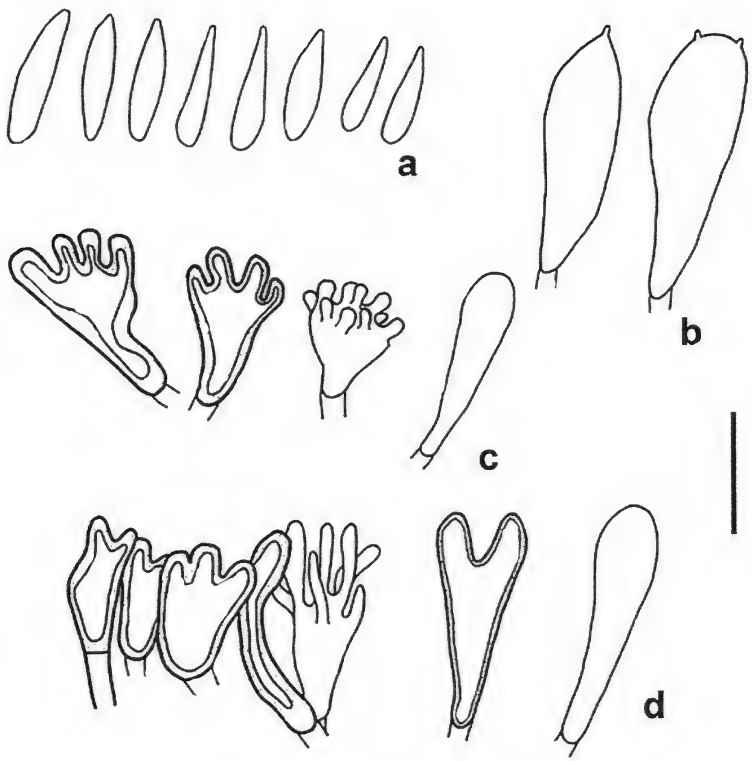

Fig. 2. *Marasmius tyrius*, micromorphology.
a: Spores. b: Basidia. c: Cheilocystidia. d: Elements of the pileipellis.
Scale bar = 10 µm.

marasmioid fungi, Singer (1976) did not present any violet-colored species with the characteristics of the subsect. *Sicciformes*, but several purple colored ones. We will mention the three most similar species. *Marasmius marthae* Singer has a purple-red pileus, black stipe at maturity, somewhat longer spores, and grows on wood pieces; *M. rubromarginatus* Dennis has a carmine-purple to brown-red pileus, more distant lamellae (11–15), and longer spores; and *M. sanguirotalis* Singer has a dark purple pileus, distant lamellae (11–13), much longer spores, and grows on branches and woody matter. Among the few other purple or purple tinged species not treated by Singer (1976), the most similar is *M. purpureobrunneolus* Henn. from Indonesia. It differs by reddish-brown to purplish-brown pileus and more distant lamellae (9–14).

The only two violaceous species in subsect. *Sicciformes* were described by Singer (1989) from Brazil, both with significantly smaller spores: *M. iodactylus* Singer (spores 6–7.5 × 2.5–3.3 µm) and *M. izonetae* Singer (spores 5 × 2.2 µm).

In sect. *Marasmius*, subsect. *Marasmius* (= *Pararotulae* Singer), Singer (1976) described *M. violeorotalis* Singer with a violet-lilac pileus. It has the same habitat as *M. tyrius* (on leaves of dicotyledonous trees) but differs in smaller spores (6.2–9.5 × 2.5–3 μm), Rotalis-type broom cells in the pileipellis, and more distant lamellae (ca. 12). Other *Marasmius* species with violaceous or violaceous tinged pileus belong to other sections of the genus.

Acknowledgments

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Arrasia rostrata (*Basidiomycota*), a new corticioid genus and species from Italy

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Abstract — An unusual corticioid species with distinctive large basidiospores that develop a distal refractive rostrum when fully mature is described as new. It grows on living bark of *Juniperus phoenicea* on the Italian island of Sardinia. Because it is morphologically distinct from any known genus of corticioid fungi, the new genus *Arrasia* is proposed to accommodate it.

Key words — dendrotheloid fungi, Italy

Introduction

Many new species of polypores and corticioids have recently been described from Sardinia: *Aleurodiscus ilexicola* Bernicchia & Ryvarden, *Antrodiella ichnusana* Bernicchia et al., *Antrodia sandaliae* Bernicchia & Ryvarden, *Echinodontium ryvardenii* Bernicchia & Piga, *Neolentiporus squamosellus* (Bernicchia & Ryvarden) Bernicchia & Ryvarden, *Phellinus juniperinus* Bernicchia & S. Curreli, and *Vararia maremmana* Bernicchia. Sardinia may be a refugium from the last glacial period, as demonstrated by the presence of *Piloporia sajanensis* (Parmasto) Niemelä, previously known as a boreal species, or the occurrence of *Echinodontium ryvardenii*, while other species of *Echinodontium* Ellis & Everh. are known from North America and Asia.

Conservation International (CI 2007) lists the Mediterranean Basin as a "Biodiversity Hotspot." Several basidiomycete species associated with Mediterranean juniper forests are *Echinodontium ryvardenii*, *Hyphoderma etruriae* Bernicchia, *Lenzitopsis oxycedri* Malençon & Bertault, *Peniophora*

junipericola J. Erikss., *Phellinus juniperinus*, *Trametes junipericola* Manjón et al., and *Vararia maremmana*. Recently, a striking corticioid species was discovered from Sardinia growing on living bark of *Juniperus phoenicea* L. (*Cupressaceae*). This new species is here fully described and illustrated; because it has no close relatives in any described corticioid genus, a new genus is proposed.

Materials & methods

For light microscopic studies, samples were mounted in 3% potassium hydroxide (KOH), Melzer's reagent (IKI), and 0.1% cotton blue in 60% lactic acid to determine cyanophily of basidiospore walls. Crystalline deposits were dissolved in a 50% HCl solution to individualize microscopical elements. Line drawings were made with a camera lucida attachment. Specimens are deposited in HUBO, CFMR, and SALA.

Taxonomy

Arrasia Bernicchia, Gorjón & Nakasone, **gen. nov.**

MycoBank MB 561760

Basidiomata effusa, adnata, tenuissima, levia, tenuiter farinosa, margine distincto. Systema hypharum monomiticum; hyphae generativae fibulatae, tenuitunicatae et ramosae. Dendrohyphidia filamentosa, ramosa, fibulata. Basidia suburniformia deinde subclavata, flexuosa, fibulata, tetraspora. Basidiosporae hyalinae, leviter crassitunicatae, leves, cyanophylae, inamyloideae, indextrinoideae, subfusoideae vel biapiculatae, parte distali se extendente ad rostrum crassitunicatum et refractivum.

Type species: *Arrasia rostrata* Bernicchia, Gorjón & Nakasone

Etymology: the genus is dedicated to Luigi Arras, mycologist and friend, who is always present during the mycological excursions across Sardinia.

Basidiomata effuse, adnate, thin, white, smooth, finely farinaceous, with a distinct margin.

Hyphal system monomitic, hyphae clamped. Dendrohyphidia filamentous, branched, clamped. Basidia suburniform at first, then flexuous, clavate to obclavate, sometimes with a basal lobe, basally clamped, with 4 sterigmata. Basidiospores broadly subfusiform to biapiculate, distal end elongating into a thick-walled rostrum, walls hyaline, slightly thickened, smooth, cyanophilous, inamyloid, nondextrinoid.

Remarks — The distinctive feature of the new genus is the rostrate basidiospores.

Arrasia rostrata Bernicchia, Gorjón & Nakasone, **sp. nov.** Plates 1–4

MycoBank MB 561761

Basidiomata resupinata, effusa, adnata, tenuissima, 30 mm longa et 5 mm lata, laevia sed farinosa vel subgausapata in maturitate, subalba vel albocinerea, margine distincto. Systema hypharum monomiticum; hyphae generativae tenuitunicatae, fibulatae, 1.8–2.3 µm latae. Dendrohyphidia filamentosa, ramosa, fibulata ad basim. Cystidia desunt. Basidia primo suburniformia deinde subclavata, flexuosa, fibulata ad basim, tenuitunicata,

Plate 1. *Echinodontium ryvardenii* (center) surrounded by circular to linear patches of *Arrasia rostrata* (Bernicchia 8087, holotype). Scale bar = 5 cm.

50–90 × 10–16 µm, tetraspora. Basidiosporae hyalinae, laeves, leviter crassitunicatae, cyanophylae, inamyloideae, indextrinoideae, late subfusoideae vel biapiculatae, parte distali se extendente ad rostrum crassitunicatum et refractivum, 27–40 × 10–15 µm. Ad corticem arborum coniferarum viventis.

Type: Italy, Sardinia, Nuoro province, Lanaittu valley, 180 m a.s.l., on bark in trunk and old branches of living *Juniperus phoenicea*, 30.III.2010, leg. A. Bernicchia, coll. 8087. **Holotype** in HUBO. **Isotype** in SALA et CFMR.

Etymology: the name *rostrata* refers to the long refractive rostrum on the basidiospores.

Basidiomata resupinate, effuse, small, circular to linear patches, becoming confluent, up to 30 × 5 mm, thin, up to 180 µm thick, soft, white, smooth, initially finely farinaceous to subfelty, finally thickly farinaceous; margin abrupt, distinct.

Hyphal system monomitic with clamped generative hyphae. Subiculum thin, a dense tissue of hyphae and crystals; subicular hyphae 1.8–2.3 µm in diam., clamped, moderately branched, walls hyaline, thin, encrusted with hyaline crystals. Subhymenium not observed. Hymenium a palisade of dendrohyphidia, basidia, and indistinct, collapsed hymenial elements obscured by crystals. Dendrohyphidia filamentous, irregular, with short branches near

apex, 40–62 × 1.5–2 µm, clamped at base, walls hyaline, thin. Cystidia absent. Basidia obclavate to suburniform at first, then flexuous, narrowly clavate to obclavate, occasionally with a lateral lobe near base, 50–90 × 10–16 µm, clamped at base, containing resinous globules, often lower part collapsed below a secondary septum, walls hyaline, thin, smooth, (2–)4-sterigmate, sterigmata stout, up to 29 × 5 µm. Basidiospores broadly subfusiform to bi-apiculate, initially distal end obtuse, later developing an extended, thick-walled, refractive rostrum or beak, 27–40 × 10–15 µm, rostrum 8–14 × 1.2–2 µm long, with a distinct, refractive, thick-walled apiculus, containing resinous material, walls hyaline, with distinct walls to slightly thick-walled, smooth, cyanophilous, not reacting in Melzer's reagent.

Habitat and distribution — Known from Sardinia growing on bark of living *Juniperus phoenicea,* frequently in association with *Echinodontium ryvardenii.*

Additional specimens examined — **ITALY. Sardinia**, Nuoro province, Lanaittu valley, 180 m a.s.l., on trunk and old branches of living *Juniperus phoenicea*, 14.XI.2009 leg. A. Bernicchia, coll. 8086, 8097; 03.II.2010, leg. L. Arras coll. 8557; 30.III.2010: leg. A. Bernicchia, coll. 7998, 8070, 8071, 8072, 8073, 8074, 8075, 8076, 8077, 8080, 8085.

Comments — The most remarkable feature of *Arrasia rostrata* is its large, beaked basidiospores. In developing basidiospores still attached to the basidium, a distal knob forms that will develop into the rostrum. When fully mature, the rostrum is a straight, thick-walled, refractive structure. By the time the basidiospore is mature and the rostrum is fully developed, the basidium is empty and collapsed. The indistinct remnants of post-mature basidia can be observed if the crystalline matter is dissolved. The apiculus of mature basidiospores is refractive and thick-walled with a notched appearance.

Arrasia rostrata is probably related to the corticioid genus *Dendrothele* Höhn. & Litsch., which shares the same ecology (inhabiting bark of living trees), crustose basidiomata, suburniform basidia, and hymenial structure with many dendrohyphidia and abundant crystalline deposits (possibly an adaptation to dry and exposed habitats). *Dendrothele* is a polyphyletic genus with species distributed among several lineages in the hymenochaetoid, russuloid, corticioid, and agaricoid clades (Goranova 2003, Goranova et al. 2003, Bodensteiner et al. 2004). The type species, *Dendrothele papillosa* Höhn. & Litsch. [= *D. griseocana* (Bres.) Bourdot & Galzin], is included in the *Niaceae* Jülich within the *Agaricales* Underw. and closely related to the cyphelloid genera *Lachnella* Fr. and *Cyphellopsis* Donk. Recently, Nakasone & Burdsall (2011) and Gorjón et al. (2011) observed navicular basidiospores in *Dendrothele* species from New Zealand and Argentina, a feature previously also known in some cyphelloid genera. Convergences in morphological traits and habit seem to have occurred repeatedly, characterizing the artificial dendrotheloid group. However, no other

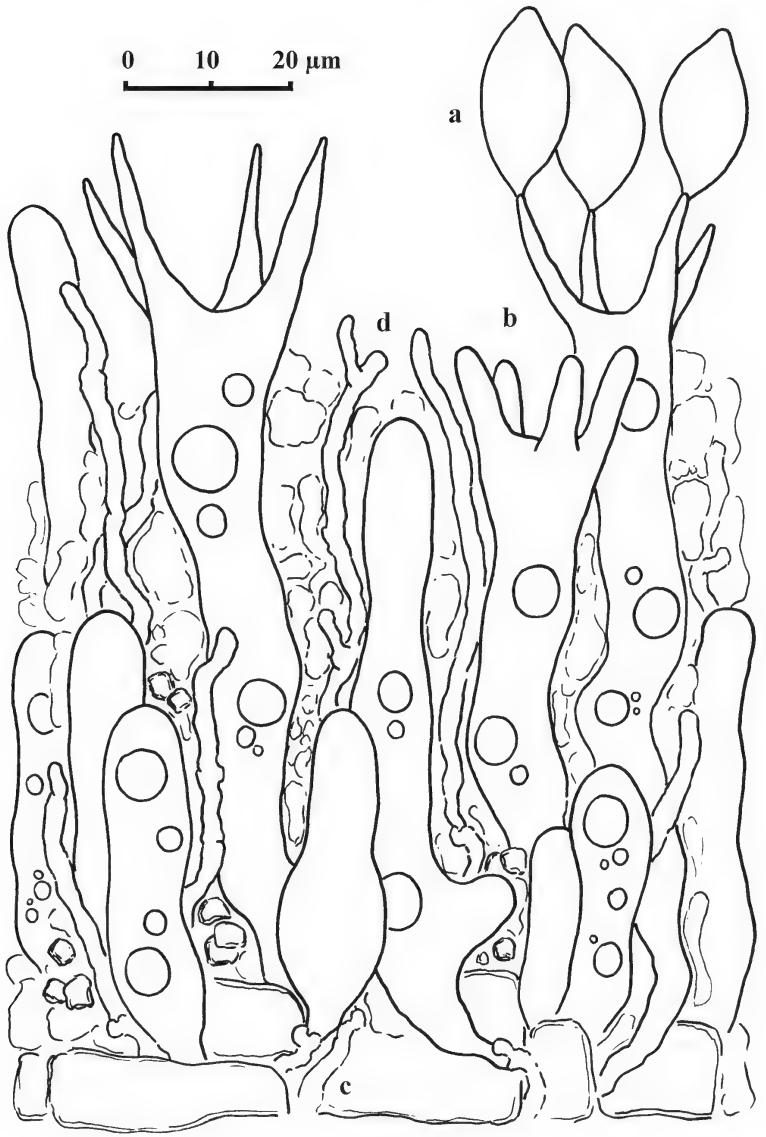

Plate 2. *Arrasia rostrata*. Hymenial elements (Bernicchia 8087, holotype).
a) immature basidiospores, b) basidia, c) generative hyphae, d) dendrohyphidia

basidiospores with a refractive rostrum are known in *Dendrothele* or any other corticioid species.

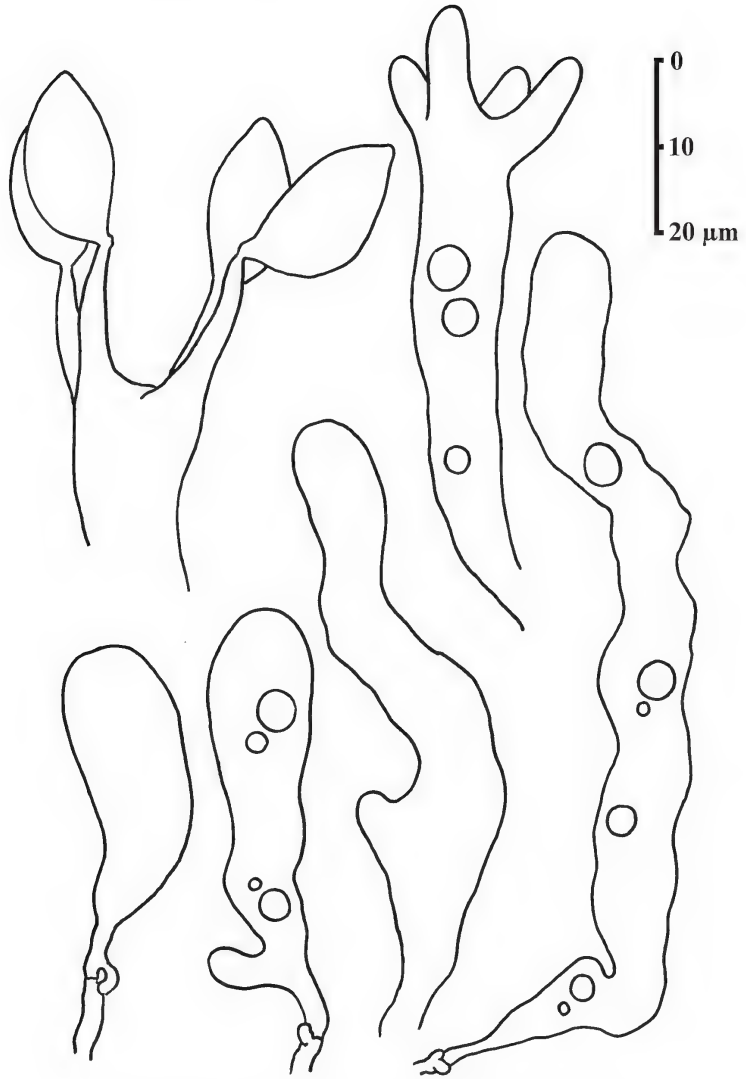

Plate 3. *Arrasia rostrata*. Basidia and immature basidiospores.
(Bernicchia 8087, holotype)

It is also interesting to note that some basidiospores in *Vararia* P. Karst. (such as in *Vararia investiens* (Schwein.) P. Karst.) develop an empty amyloid part separated by a septum in the proximal region (close to the sterigma attachment). The biological function, if any, of this structure is unknown.

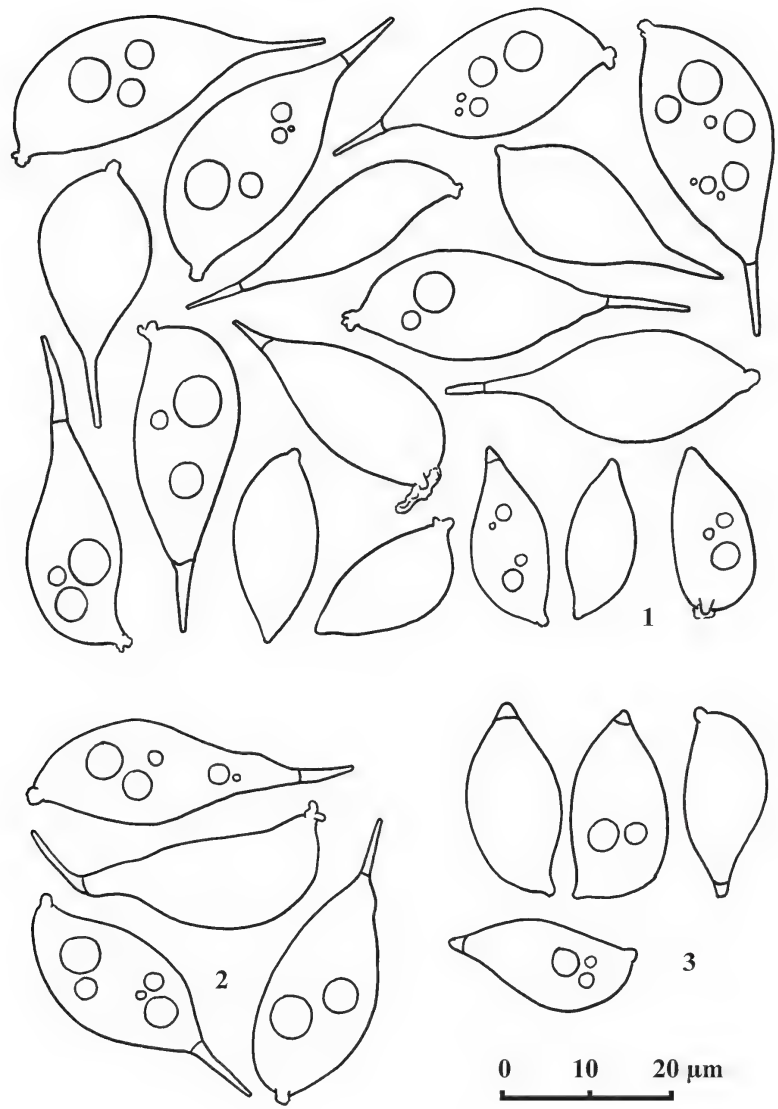

Plate 4. *Arrasia rostrata.* Mature basidiospores with a well developed rostrum and immature (right and center right) basidiospores. (1: Bernicchia 8087, holotype; 2: Bernicchia 8074; 3: Bernicchia 8071)

Morphological similarities may also be drawn with some genera belonging to *Vuilleminiaceae* Maire, *Punctulariaceae* Donk, and *Corticiaceae* Herter. Among the *Vuilleminiaceae, Vuilleminia* Maire, *Australovuilleminia* Ghobad-Nejhad & Hallenb., and *Cytidia* Quél. all share the saprophytic habit growing on attached recently dead angiosperm wood (not known from coniferous substrata), basidiomes that are usually gelatinous and decorticating, and allantoid to ellipsoid basidiospores. Members of the *Punctulariaceae*, *Punctularia* Pat., *Punctulariopsis* Ghobad-Nejhad, and *Dendrocorticium* M.J. Larsen & Gilb., grow on fallen angiosperm wood and all species have ellipsoid basidiospores. Genera in the *Corticiaceae*, as delimited by Ghobad-Nejhad et al. (2010), show a more ecological and morphological complexity, but none display the features that characterize *Arrasia rostrata.* Preliminary molecular studies indicate that *Arrasia rostrata* fits no existing homobasidiomycete genus or order thus far recognized (data not shown); further analyses are still required.

Acknowledgments

Nils Hallenberg and Alina G. Greslebin acted as presubmission reviewers and their comments are acknowledged. We thank Luigi Arras and Marco Facchini for their help, Giovanni Consiglio for revision of Latin diagnosis, and Cristina Spinelli for the colour photo. The Council of Lanusei (Sardinia, Italy) supported AB and SPG on some collecting trips. Karl-Henrik Larsson and Ellen Larsson sequenced *Arrasia rostrata* and conducted a preliminary molecular analysis, and we are very grateful for their comments about the molecular relationship of the new species.

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Cortinarius mikedavisii sp. nov. from northern California

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Abstract — A new *Cortinarius* (subg. *Phlegmacium*) species is described from northern California. *Cortinarius mikedavisii* is a brightly colored bulbopodium in sect. *Laeticolores*. Phylogenetically it belongs in the /cupreorufus clade.

Key Words — *Cortinariaceae*, fungal taxonomy, nrITS data

Introduction

Cortinarius sect. *Laeticolores* M.M. Moser ex Moënne-Locc. & Reumaux was erected to accommodate some of the brightly colored bulbopodiums of subg. *Phlegmacium*. The section type was originally designated as "*Cortinarius orichalceus*" (a misapplied name, not conspecific with *Agaricus orichalceus* Batsch); its valid name is *Cortinarius cupreorufus* Brandrud (Brandrud et al. 1994).

Recent molecular studies (Frøslev et al. 2007, Garnica et al. 2009), congruent with my own phylogenetic analyses, show that the broad circumscription of sect. *Laeticolores* by Moser (1960) and Bidaud et al. (2004) is polyphyletic. However, a group of species around *C. cupreorufus* forms a well-supported clade including *C. rufo-olivaceus* (Pers.) Fr. and *C. prasinus* (Schaeff.) Fr. The /cupreorufus clade, which represents the core of sect. *Laeticolores*, is well represented in western North America with *Cortinarius mikedavisii*, described here as new, and other undescribed species (Fig 1).

Materials & methods

Methods for morphological studies and DNA extraction, PCR conditions and primers, PCR product clean up, and sequencing were employed as outlined in Bojantchev & Davis (2011). Color codes follow Munsell™ soil color charts (Anonymous 2000). Collections are stored in the private herbarium of the first author or at the University of California herbarium in Berkeley (UC) where noted.

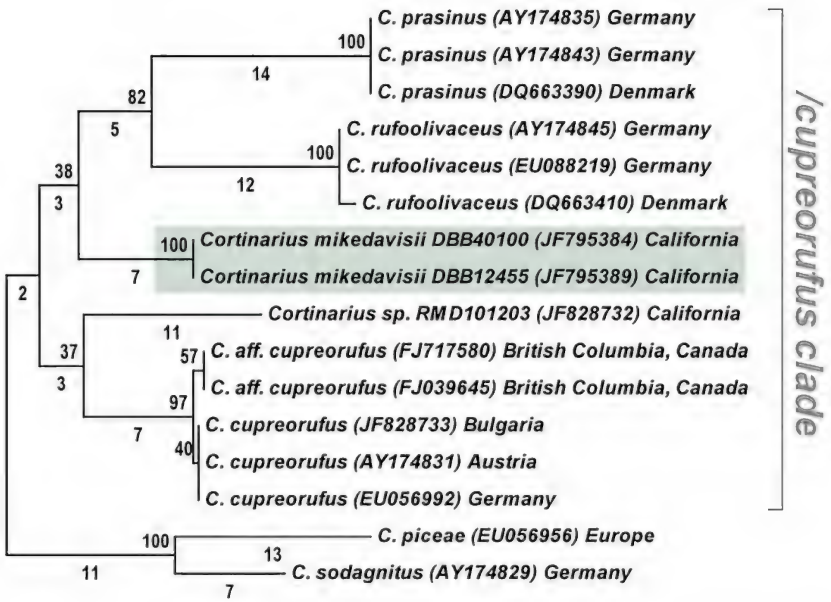

FIG 1. Phylogenetic tree inferred by maximum parsimony analysis of 16 *Cortinarius* subg. *Phlegmacium* nrITS sequences. The tree shows the position of *C. mikedavisii* relative to its closest neighbors in the /cupreorufus clade. The percentages of clustered replicate trees based on the bootstrap test (1000 replicates) are shown above the branches while the branch lengths representing estimated nucleotide substitutions are shown below. GenBank accession numbers are enclosed in brackets.

PHYLOGENETIC ANALYSIS—During these studies all *Cortinarius* nrDNA sequences from the public databases GenBank (http://www.ncbi.nlm.nih.gov) and UNITE (http://unite.ut.ee/) were downloaded and reviewed. Preliminary phylogenetic analysis (not shown) of 1012 *Phlegmacium* nrITS sequences from the northern hemisphere, including 312 sequences from the author's own collections, clarified the closest relatives of *C. mikedavisii* and resolved its position within the /cupreorufus clade of the calochroid super-clade as defined by Frøslev et al. (2007) and Garnica et al. (2009).

A set of sixteen *Phlegmacium* sequences representing eight taxa was selected for a detailed phylogenetic analysis. Twelve sequences were sourced from GenBank and four sequences are from the author's collections. Fourteen sequences were from the /cupreorufus clade, represented by three well known European taxa combined with *C. mikedavisii* and two western North American taxa close to *C. cupreorufus*. *Cortinarius sodagnitus* Rob. Henry and *C. piceae* Frøslev et al. {formerly known as *C. calochrous* var. *coniferarum* (M.M. Moser) Nezdojm.} were selected as the outgroup because they represent the core of the calochroid cortinarii but are distant from the /cupreorufus clade.

Sequence alignments were generated with MAFFT v6.821b (Katoh et al. 2002) with the G–INS–i global alignment iterative refinement strategy. Minimal gap opening and extension penalties were set for better resolution of the more variables sectors within the nrITS. The alignments were visually inspected and corrected where needed.

The evolutionary history was inferred using the Maximum Parsimony method as implemented by MEGA5 (Tamura et al. 2007). The MP trees were generated by the Close-Neighbor-Interchange algorithm with search level 0 in which the initial trees were obtained with the random addition of sequences (10 replicates). The percentage of replicate trees in which the associated taxa clustered together was calculated from a bootstrap test with 1000 replicates. The search resulted in thirty most parsimonious trees (length = 97), which differed mainly in the topology of the terminal nodes. One tree is given in Fig. 1.

Taxonomy

Fig 2. *Cortinarius mikedavisii* (UC 1860820, holotype).

Cortinarius mikedavisii Bojantchev, **sp. nov.** Figs 2–6

MycoBank MB 561221

Pileo 60–220 mm lato, hemispherico, dein planoconvexo, planoconcavo, glutinoso, roseobrunneo, cum KOH purpureobrunneo, margine involuto. Lamellis emarginatis, caesiis dein flavidis, purpureobrunneis, in statu senili. Stipite 50–140 mm longo, cylindrico, bulbo marginato, 30–50 mm lato. Velo universale roseobrunneo. Velo partiale copioso, flavido. Carne caesio dein albido. Sapore miti. Sporis 10–13 × 6–7 µm, amygdaliformibus, verrucosis, basidiis 28–42 × 8–9 µm, tetrasporigeris, fibulis praesentibus.

Type: USA. California: Mendocino County, Caspar, Caspar Cemetery, under *Picea sitchensis*, 27 Nov 2010, Bojantchev DBB40100 (**Holotype** UC 1860820; Genbank nrITS JF795384).

Etymology: In honor of Prof. Mike Davis whose encouragement and support in the area of molecular genetics made these *Cortinarius* studies possible.

Stature pileocarpous bulbopodium. Pileus 60–220 mm diam., hemispherical to convex when young, plano-convex to plano-concave at age; margin involute then straight; colors intense, mostly reddish to orange brown on the disk, varying from carmine brown to rose brown (10R 3/6–4/8), paler on the margin, copper brown to sulphur yellow (10R 6/8–7.5YR 7/8); surface very glutinous when wet, glabrous to dull glossy when dry. Lamellae crowded, 10–22 mm broad, blue (GLEY2 6/10G–8/10G) at first, turning olive (5Y 6/4–8/4) then yellowish clay (5Y 8/6–8/8) with purplish tinges, then purplish brown as the spores mature; edge even; attachment sinuate; lamellulae abundant. Stipe 50–140 mm long, 15–30 mm wide, cylindrical to subclavate above the bulb, pale yellow, with rose brown streaks from universal veil. Bulb 30–80 mm diam at the widest point, well-developed, abruptly emarginate, tapering below, rose to purplish brown around margin; the subterrestrial part with a pale to sulphur yellow cottony mycelial felt and rhizomorphs. Universal Veil rose to purplish brown, leaving copious remnants on the pileus and bulb margin. Cortina yellow, leaving an annular zone of dense fibrils on the stipe. Context white to bluish at first, paling to white at maturity with yellow tinges in the bulb. Odor earthy. Taste mild, earthy. Macrochemical Reactions 5% KOH strong and complex, dark purple red on the pileus; on the context pale olive at first, soon lilac on pileus context, strongly purplish on bulb context and paler on stipe context. Spore Deposit deep rusty brown.

Basidiospores (9.5–)10.5–13.2(–15.5) × (5.7–)6.2–7.2(–7.8) µm (mean 11.5 × 6.7 µm), Q = 1.61–1.78, Q_{av} = 1.72 (N = 178, 7 basidiomata, four collections), amygdaliform, some with apical papilla, strongly verrucose. Basidia 28–42 × 8–9 µm, 4-spored, cylindro-clavate, clamped. Gill edge sparsely fertile.

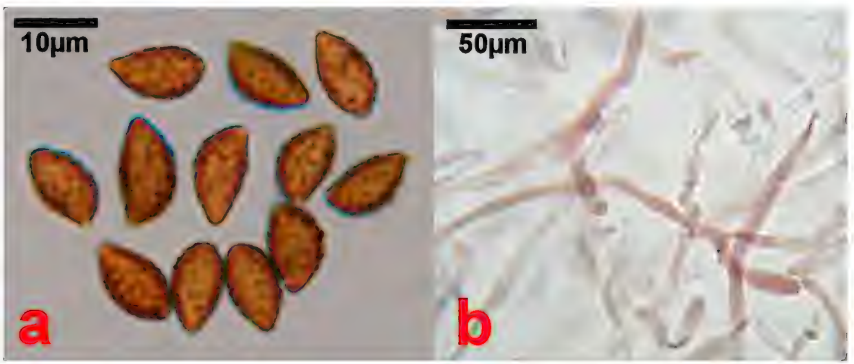

Fig 3. *Cortinarius mikedavisii*. a) Basidiospores (UC 1860820, holotype) b) Cuticle hyphae with an olive to purplish intracellular pigment in 5% KOH (UC 1860820, holotype)

Fig 4. *Cortinarius mikedavisii.* 5% KOH reaction a) UC 1860820, holotype b) DBB12455

Cystidia not observed. Pileipellis an ixocutis, simplex, no hypodermium detected, composed of parallel to interwoven hyphae in a dense gelatinous matrix 230–300 µm thick, made up of 4–12 µm wide, irregular hyphae, with greenish to purplish intracellular pigment when mounted in 5% KOH. No distinct reactions to Melzer's reagent were observed. Clamp connections common in all parts.

Habitat and distribution — *Cortinarius mikedavisii* appears to be an uncommon species. Currently it is known from only three locations, at two of which it fruits regularly. The habitat is mixed woods with Sitka spruce (*Picea sitchensis* (Bong.) Carrière) being the one common symbiont in all locations. The author has never seen it south of Mendocino. Its distribution appears to be limited to northern California and southern Oregon, where it was collected once. There are no matching records from Washington and British Columbia despite the intensive collecting and molecular cataloguing that has taken place in these regions.

Additional collections examined: **USA. California:** Mendocino County, Caspar, Caspar Cemetery, under *Picea sitchensis,* 23 Dec 2008, Bojantchev DBB12455, (UC 1860821, Genbank nrITS JF795389); Jackson State Demonstration Forest, under *Picea sitchensis,* 22 Nov 2009, Bojantchev DBB28043; **Oregon:** Curry County, Samuel Boardman State Park, under *Picea sitchensis,* 11 Nov 2009, Bojantchev DBB27700.

Discussion

Cortinarius mikedavisii is a beautiful species that so stands out with its large size and bright colors that it should present no difficulty for field identification. The distinctive alkaline color reaction indicates the presence of rufoolivacin, an anthraquinone compound, which Steglich & Oertel (1985) detected in other members of the /cupreorufus clade.

Phylogenetically, *C. mikedavisii* (Fig 1) stands almost equidistant in the clade between the two main branches of *C. cupreorufus* and *C. rufo-olivaceus*. *Cortinarius rufo-olivaceus,* a beech associate from Europe, is not known from

Fig 5. *Cortinarius mikedavisii.* a) Mature basidiomata can become very large (DBB12455). The scale is a US quarter dollar coin (~25mm) b) Basidiomata in a button stage (DBB28043)

Fig 6. *Cortinarius mikedavisii* — a study in the development of a pileocarpous bulbopodium. Note the changing color of the gills. a) DBB28043 b) DBB28043 c) DBB27700

Fig 7. *Cortinarius mikedavisii.* a) DBB12455. The scale is a US quarter dollar coin (~25mm) b) Yellow mycelial strands (DBB27700)

western North America. *Cortinarius cupreorufus* (Fig 8a) and its western American ally differ in the straw yellow lamellae and generally more pale olive pileus, colors noticeable in all stages of development. The alkaline reactions in *C. mikedavisii* are more intense than seen in *C. cupreorufus,* and the context turns lilac to purplish red immediately, bypassing the pale olive stage. There is

Fig 8. Related species: a) *Cortinarius cupreorufus* DBB19840 from Europe; b) *Cortinarius* sp. RMD101203, an undescribed species from the xeric oak stands of central and southern California.

an undescribed species in the same clade, represented by coll. "RMD101203" in Fig. 1, which is only found in xeric oak stands of the central valley and southern California and differs significantly in colors (Fig. 8b). All members of the /cupreorufus clade share large (10–13 µm) amygdaloid spores with very rough ornamentation.

Complete iconography of *C. mikedavisii* and a comparative image study is available on http://www.mushroomhobby.com.

Acknowledgements

The author is grateful to Dr. Else C. Vellinga and Dr. Boris Assyov for their presubmission reviews and comments. Dr. Shaun Pennycook was very helpful in answering nomenclatural questions during the preparation of this manuscript. Boris Assyov reviewed and corrected the Latin diagnosis.

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Observations on gasteroid *Agaricomycetes* from the Brazilian Amazon rainforest

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Abstract — Field trips carried out in an indigenous protected area in the states of Rondônia and Mato Grosso (Brazil) in 2009 revealed new records: *Geastrum albonigrum,* new from South America; and *Geastrum lageniforme*, *Mutinus caninus,* and *Tulostoma exasperatum*, new from the Brazilian Amazon rainforest. Descriptions, photographs, and line drawings of the specimens are presented.

Key words — *Basidiomycota*, fungal taxonomy, gasteromycetes, neotropical mycota

Introduction

The Amazon rainforest is recognized as one of the areas with higher biodiversity of the globe. Interesting data on macrofungal taxonomy from the Brazilian Amazon rainforest have been published recently by Desjardin & Braga-Neto (2007), Martins-Júnior et al. (2008), Gibertoni (2008), Gibertoni et al. (2008), Gomes-Silva et al. (2008, 2009, 2010a,b, 2011), Sotão et al. (2008), Gomes-Silva & Gibertoni (2009), Trierveiler-Pereira et al. (2009a), and Baltazar et al. (2010). However, gasteromycete diversity is poorly known in this Brazilian ecosystem.

Although more than two hundred species of gasteroid fungi have been acknowledged from Brazil (Trierveiler-Pereira & Baseia 2009), fewer than fifteen species are known to occur in the Amazon rainforest, an unexplored highly diverse area.

Species of gasteroid fungi previously recorded from the Brazilian Amazon forest are listed in Trierveiler-Pereira et al. (2009a), including the description of the new species, *Cyathus amazonicus* Trierveiler-Pereira & Baseia.

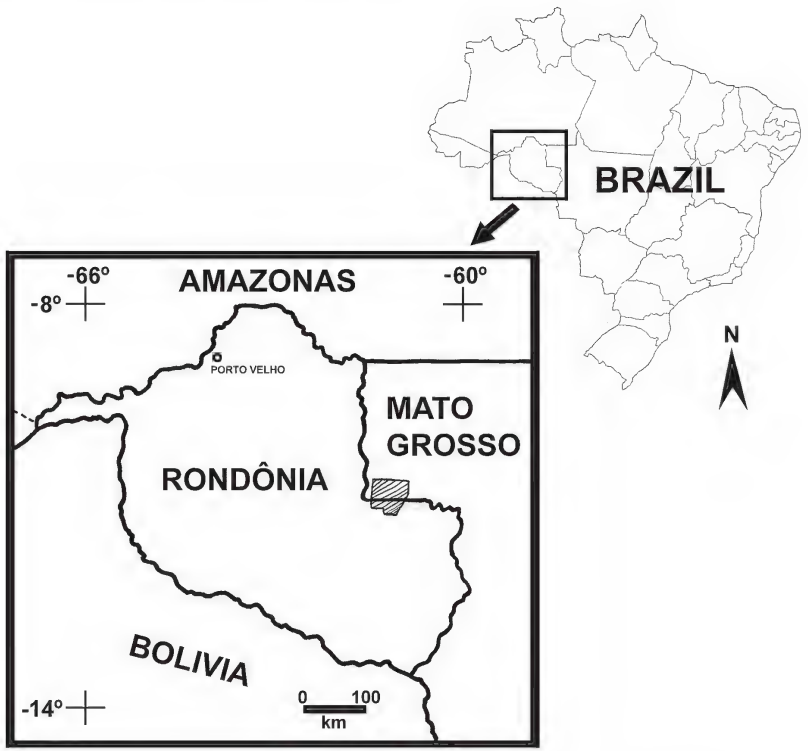

Fig. 1. Location of the 'Terra Indígena Sete de Setembro' (hatched area) in the states of Rondônia and Mato Grosso (Brazil).

Materials & methods

Fungi were collected along pre-existing trails in a Brazilian indigenous protected area called 'Terra Indígena Sete de Setembro' (TISS). This territory shelters ten indigenous tribes distributed in the states of Rondônia (RO) and Mato Grosso (MT) (Fig. 1). In this area there are about 1200 indigenous individuals from the ethnic group of Paiter-Suruí. The TISS extends over approximately 249 thousand hectares and its territory is mostly covered by the Amazonian dense ombrophilous forest (Radambrasil 1978, Ferronato & Nunes 2010).

Field trips were carried out from May 28th to June 06th 2009 in the tribal areas 'Sertanista Apoena Meirelles' (Rondolândia, MT; 10°10′15″S 59°28′14″W) and 'Lapetanha' (Cacoal, RO; 11°26′19″S 61°26′50″W).

The collected basidiomata were placed in paper bags or plastic containers prior to analysis. Macroscopic descriptions are based on observations of fresh and dried material (Miller & Miller 1988). Colors are coded according to Kornerup & Wanscher (1978). Microscopic observations of tissues from dried specimens mounted in 5% KOH were made under a light microscope.

Voucher specimens were deposited in the Herbarium HFSL and duplicates were sent to Herbarium URM. Additional specimens deposited in MA-Fungi, ICN, and MBM were also examined. Gasteromycete specimens cited in Capelari & Maziero (1988) were borrowed from the Herbarium SP to confirm identification. Herbarium acronyms are according to Thiers (2011).

Taxonomy

Geastrum albonigrum Calonge & M. Mata, Bol. Soc. Micol. Madrid 28: 332. 2004. FIG 2, 4A

SPECIMENS EXAMINED: **BRAZIL. MATO GROSSO**, RONDOLÂNDIA, Terra Indígena Sete de Setembro, Aldeia Apoena Meirelles, 30.V.2009, leg. Gomes-Silva et al. 209 (URM 82098).

IMMATURE BASIDIOMATA epigeous, globose to ovoid, 2.1 cm high × 1.5 cm broad, grayish brown (4C5) to yellowish brown (5F5), hirsute, with a single robust rhizomorph attached at the base, rhizomorph ramified or not, 0.6–1.8 cm length, with debris strongly attached. EXPANDED BASIDIOMATA 0.7–1.9 cm high × 2–3.4 cm broad. EXOPERIDIUM non-hygroscopic, split into 6–7 rays, saccate or with rays becoming involute, more rarely arched; external layer hirsute, grayish brown (4C5) to yellowish brown (5F5), peeling off at maturity; fleshy layer persistent, brown (6E5) to grayish brown (6E3), with a purplish shade. ENDOPERIDIUM globose to depressed globose, 0.7–1.6 cm high × 1.2–1.6 cm broad, brown (6E6), sessile, without apophysis; peristome fibrillose, not delimited, slightly darker than endoperidium. GLEBA pulverulent at maturity, dark brown (6F4).

BASIDIOSPORES globose, 4–4.5 µm diam. (including ornamentation), yellowish brown to dark brown in KOH, ornamented with short columns. CAPILLITIAL HYPHAE straight, thick-walled, with narrow lumen, pale brown to yellowish brown in KOH, 3–6 µm diam., not branched.

ECOLOGY & DISTRIBUTION: Gregarious on rotten wood, without subiculum. The Brazilian material represents the first record from South America. Previously known from Costa Rica, Mexico, and Panama. It is possible that the species occurs in neotropical rainforests and warmer regions of temperate America.

ADDITIONAL SPECIMENS EXAMINED: **COSTA RICA. GUANACASTE**, TEMPISQUE, Parque Nacional Palo Verde, 27.IX.2003, leg. I. López 4869 (MA-Fungi 59260, **isotype**); 20.X.2004, leg. I. López 6277 (MA-Fungi 65423); **MEXICO. CHIAPAS**, Tapachula, 03.X.1993, leg. G. Guzmán 30743 (MA-Fungi 59258); **PANAMA. ISLA DE COIBAS**, Cerro de la X, 14.XII.1996, leg. Pando & Núñez (MA-Fungi 36140-2).

REMARKS: *Geastrum albonigrum* is characterized by an epigeous hirsute form when young, a saccate or involute exoperidium that peels off in age, a sessile endoperidium, and a non-delimited fibrillose peristome. When the dark hirsute external exoperidial layer peels off, it reveals a white fibrous layer

where the rhizomorph remains attached. The fleshy layer is purplish and the endoperidium is dark.

Brazilian material differs from the holotype in spore measurements (3–5 µm in the original description) and the presence of a narrow lumen in the capillitial threads (absent in the material described by Calonge & Mata 2004).

Geastrum albonigrum belongs to the group of epigeous xylophilous *Geastrum* species, such as *G. schweinitzii* (Berk. & M.A. Curtis) Zeller, *G. hirsutum* Baseia & Calonge, and *G. javanicum* Lév., all of which differ by forming a white subiculum over the substrata (Ponce de León 1968, Baseia et al. 2003, Baseia & Calonge 2006), a character not seen in *G. albonigrum*.

Geastrum hirsutum also has a hirsute exoperidium that peels off at maturity, but lacks a robust rhizomorph, has a delimited peristome, and a light-colored endoperidium and fleshy layer. Similar to *G. hirsutum*, *G. schweinitzii* produces smaller basidiomata lacking a hirsute external layer. *Geastrum javanicum* has no hirsute external layer and it rarely peels off completely (Trierveiler-Pereira et al. 2011).

Geastrum lageniforme Vittad., Monogr. Lycoperd.: 16. 1842. FIG 3A, 4B

SPECIMENS EXAMINED: **BRAZIL. MATO GROSSO**, RONDOLÂNDIA, Terra Indígena Sete de Setembro, Aldeia Apoena Meirelles, 30.V.2009, leg. Gomes-Silva et al. 206, 222 (URM 82099, 82100).

IMMATURE BASIDIOMATA not observed. EXPANDED BASIDIOME 0.7–1.0 cm high × 2.3–3.0 cm diam. EXOPERIDIUM non-hygroscopic, split into 6–8 rays, saccate, rays long, slender, external layer glabrous, with longitudinal ridges, grayish yellow (1B5) to blond (4C4); fleshy layer persistent, brownish gray (6C2). ENDOPERIDIUM globose, 0.7–0.9 cm high × 1.0–1.2 cm broad, grayish beige (4C2) to brownish gray (5D2), sessile, without apophysis; peristome fibrillose, grayish brown (5E3), delimited by a whitish line. GLEBA pulverulent at maturity, brownish gray (5E2).

BASIDIOSPORES globose, 3.5–5 µm diam. including the ornamentation, yellowish brown in KOH, ornamentation columnar. CAPILLITIAL HYPHAE straight to more or less sinuous, slightly thick-walled, with narrow lumen, yellowish in KOH, 2.5–8.0 µm diam., not branched.

ECOLOGY & DISTRIBUTION: Solitary on forest soil. Widespread (Calonge et al. 2004). In Brazil, the species has been reported from the states of Rio Grande do Sul (Rick 1961, Cortez et al. 2008), Rio de Janeiro (Hennings 1904), Bahia (Trierveiler-Pereira et al. 2009b), and Pernambuco (Trierveiler-Pereira et al. 2011). This is the first record from the Brazilian Amazon rainforest.

ADDITIONAL SPECIMEN EXAMINED: **SPAIN. BURGOS**, QUINTANA DEL PIDIO, 22.XI.1991, leg. L.A. Parra (MA-Fungi 30752).

REMARKS: *Geastrum lageniforme* resembles *G. saccatum* Fr. in a saccate exoperidium, sessile endoperidium, and fibrillose delimited peristome (Trierveiler-

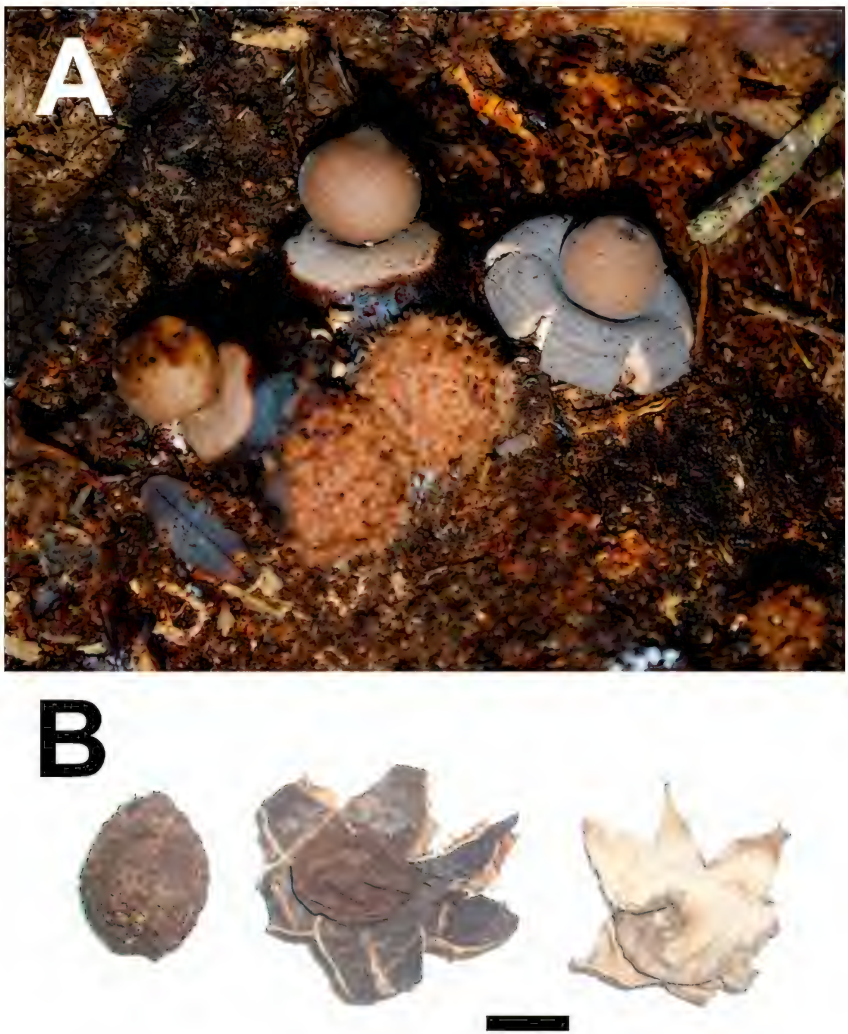

Fig. 2. *Geastrum albonigrum*.
A. Mature and immature basidiomata in situ.
B. Basidiomata in different stages of development.

Pereira et al. 2011). According to Sunhede (1989), the two species can be distinguished by the presence of clamped hyphae in the external mycelial layer, which occur only in *G. lageniforme*.

Ponce de León (1968) considered *G. lageniforme* a synonym of *G. indicum* (Klotzsch) Rauschert (= *G. triplex* Jungh.), but there are characteristics that can separate these two species, since *G. triplex* is usually larger, with involute rays, and forms a fleshy collar around the endoperidium.

Mutinus caninus (Huds.) Fr., Summa Veg. Scand. 2: 434. 1849. Fig 3c, 4c

Specimen examined: **BRAZIL. Rondônia**, Cacoal, Terra Indígena Sete de Setembro, Aldeia Lapetanha, 05.VI.2009, leg. Gomes-Silva et al. 126 (URM 82101).

Basidiome 5.5 cm high, with a whitish rhizomorph attached at the base, rhizomorph ramified, up to 2.2 cm long. Volva saccate, 1.6 cm high × 1 cm broad, yellowish white (1A2). Pseudostipe cylindrical, tapering at the apex, 4.1 cm high × 0.7 cm broad, spongy, light orange (5A4) at the base and becoming reddish towards the apex, fertile region reddish orange (7B8). Gleba mucilaginous, olive brown (4F5), covering the fertile region.

Basidiospores cylindrical, 4.5–5.5 × 1.5 µm, hyaline to greenish in KOH, smooth, biguttulate.

Ecology & distribution: Solitary on forest soil. Cosmopolitan, but rare in the tropics (Baseia et al. 2006). In Brazil the species was reported only from Rio Grande do Norte (Baseia et al. 2006). This is the first record from the Brazilian Amazon rainforest.

Additional specimens examined: ***Mutinus caninus*** – **SPAIN. Guipuzcoa**, San Sebastián, 16.X.1976, leg. Sociedad de Ciencias Naturales Arazandi (MA-Fungi 22361). ***Mutinus elegans*** – **SPAIN. Lugo**, Chantada, 03.XI.1999, leg. M.T. Jacobo s/n (MA-Fungi 42064); **BRAZIL. Rio Grande do Sul**, Viamão, Parque Estadual de Itapuã, 22.V.2004, leg. V.G. Cortez 016/04 (ICN 139004).

Remarks: *Mutinus caninus* is similar to *M. elegans* (Mont.) E. Fisch. but forms a well delimited gleba on the apical zone; the glebal zone is not defined in *M. elegans* (Calonge 1996). The pseudostipe in the examined material is yellowish, but according to Calonge (1996) it may also be whitish or pinkish. Zeller (1944) described a white variant of this species, *M. caninus* var. *albus* Zeller.

Tulostoma exasperatum Mont., Ann. Sci. Nat., Bot., Sér. 2, 8: 362. 1837. Fig 3b, 4d

Specimens examined: **BRAZIL. Rondônia**, Cacoal, Terra Indígena Sete de Setembro, Aldeia Lapetanha, 06.VI.2009, leg. Gomes-Silva et al. 100, 145 (URM 82102, 82103).

Basidiomata 1.1–7.0 cm high. Spore sac globose to depressed-globose, 0.6–0.8 cm high × 1.8–2.2 cm broad. Exoperidium spiny, light brown (5E7), peeling off at maturity. Endoperidium reticulate, papery, yellowish white (2A2) to pale yellow (4A3); peristome conical, slightly lighter than endoperidium, fibrillose, delimited. Gleba dull yellow (3B3). Stipe 0.9–6.1 cm high × 0.2–0.25 cm diam., light brown (5E7), with longitudinally arranged scales.

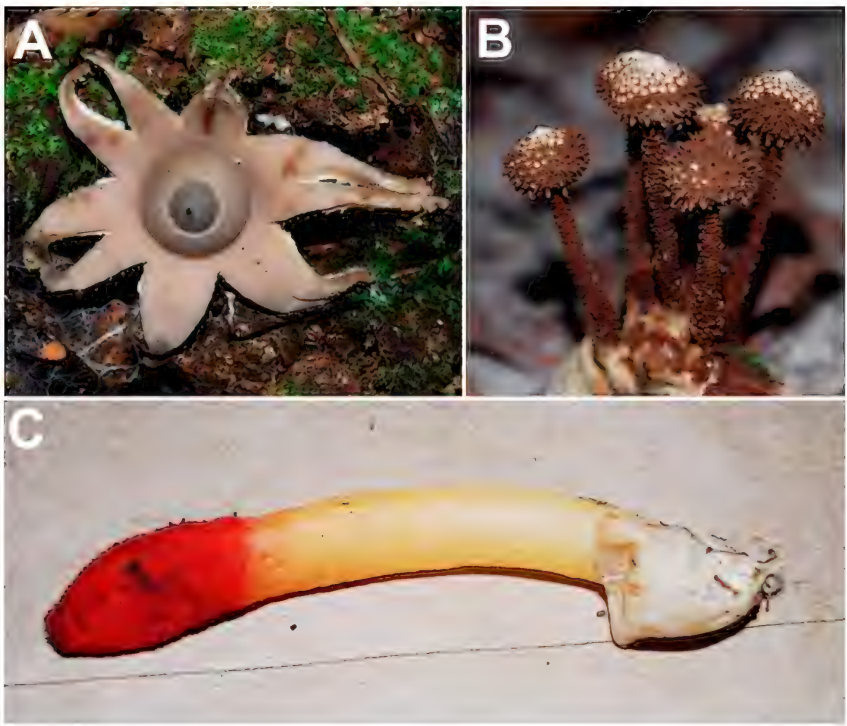

Fig. 3. Gasteroid species from the Brazilian Amazon rainforest.
A. *Geastrum lageniforme*. B. *Tulostoma exasperatum*. C. *Mutinus caninus*.

Basidiospores globose to subglobose, 6–7.5 µm diam., yellowish in KOH, with a columnar-reticulate ornamentation. Capillitial hyphae straight to tortuous, thick-walled, swollen at the septa, branched, light yellow in KOH, 4–7 µm diam.

Ecology & distribution: Gregarious on rotten wood. Widely distributed in tropical and subtropical regions (Wright 1987). In Brazil the species is known from Rio Grande do Sul (Rick 1961, Cortez et al. 2009), Paraná (Meijer 2006), Paraíba (Silva et al. 2007), São Paulo and Pernambuco (Baseia & Milanez 2002). This is the first record from the Brazilian Amazon rainforest.

Additional specimen examined: **BRAZIL. Rio Grande do Sul**, Viamão, Parque Estadual de Itapuã, 25.VI.2005, leg. R.M. Silveira 456 (ICN 154635); **Paraná**, Tibagi, Quartela, 06.IX.1992, leg. A.A.R. de Meijer 2341 (MBM).

Remarks: Among the 14 *Tulostoma* species recorded from Brazil (Baseia & Milanez 2002, Meijer 2006, Silva et al. 2007, Cortez et al. 2009), *T. exasperatum* is the only one with a lignicolous habit. *Tulostoma* species usually occur on sandy soil, pastures, humus, forest soil and even flooded areas (Wright 1987).

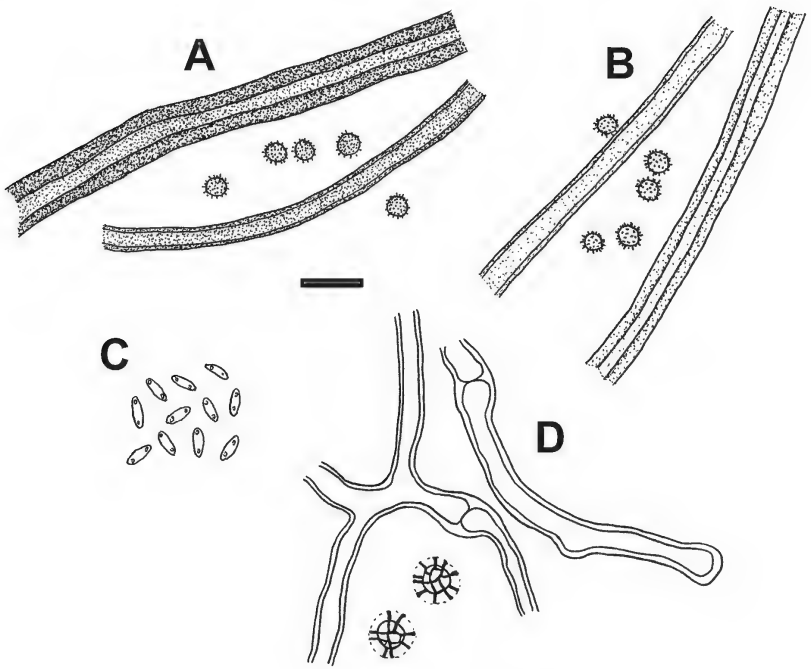

Fig. 4. Line drawings of microscopic elements.
A. *Geastrum albonigrum*: capillitial threads and spores.
B. *G. lageniforme*: capillitial threads and spores. C. *Mutinus caninus*: spores.
D. *Tulostoma exasperatum*: capillitial threads and spores (scale bar = 10 µm).

Specimens kept in SP

We reviewed and confirmed the identities of the following gasteromycetes previously reported from the Brazilian Amazon rainforest by Capelari & Maziero (1988): SP 211544, SP 211545, SP 211547, and SP 211548 as *Cyathus montagnei* Tul. & C. Tul.; SP 214636 as *C. limbatus* Tul. & C. Tul.; and SP 211223 and 211526 as *Morganella fuliginea* (Berk. & M.A. Curtis) Kreisel & Dring. The immature basidiomata in SP 211549 could not be identified morphologically. We did not review SP 211527 (labelled as *Morganella* sp.) or SP 211301 (labelled as *Lycoperdon* sp.).

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Septobasidium saurauiae sp. nov. (*Septobasidiaceae*) and *S. pseudopedicellatum* new to China

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Abstract—A new species, *Septobasidium saurauiae* on *Saurauia tristyla* associated with *Chionaspis* sp., and a new Chinese record, *Septobasidium pseudopedicellatum* on *Schefflera octophylla* and *Psychotria serpens*, are described. They were collected from Hainan Province.

Key words—*Pucciniomycetes*, *Septobasidiales*, taxonomy

Hainan Island is located in the South China Sea. It is a tropical area with a rich fungal diversity. To date, six new species and one new Chinese record of *Septobasidium* have been discovered from this Province (Chen & Guo 2011b, Lu & Guo 2009a, 2010b,c). An additional new species and a new Chinese record of *Septobasidium* are reported as follows:

Septobasidium saurauiae S.Z. Chen & L. Guo, **sp. nov.** Figs. 1–7

MycoBank MB 561871

Basidiomata resupinata, discontinue crescentia, 6–7 cm longa, 3–4 cm lata, cinnamomeo-brunnea vel griseo-brunnea, margine determinata, superficie laevia, saepe protuberationibus rotundis praedita, in sectione 900–1330 µm crassa. Subiculum brunneum, 30–50 µm crassum, ab subiculo stratum hypharum vel curtam columnam formans. Columnae brunneae, 20–50 µm altae, 20–70 µm latae. Strata hyphararum 780–970 µm alta. Ab strato hymenii hyphae saepe repullulantes tum stratum hypharum et hymenium secundum formantes. Stratum hypharum secundum 200–240 µm altum. Hymenium hyalinum, 40–50 µm crassum. Basidia cylindrica, recta vel leviter curvata, 4-cellularia, 42–50 × 6–8 µm, hyalina. Sine probasidio. Basidiosporae 10–25 × 4–6 µm, hyalinae. Haustoria hyalina, ex hyphis irregulariter spiralibus constantia.

Type: China, Hainan Province, Limu Mountain, alt. 529 m, on *Saurauia tristyla* DC. (*Actinidiaceae*), associated with *Chionaspis* sp. (*Diaspididae*), 23.XI.2010, Y.F. Zhu & F. He 520 (**holotype**, HMAS 263145).

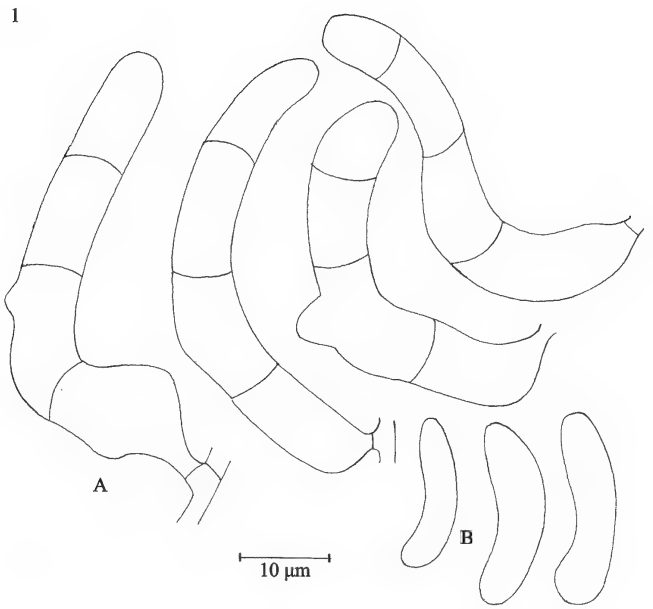
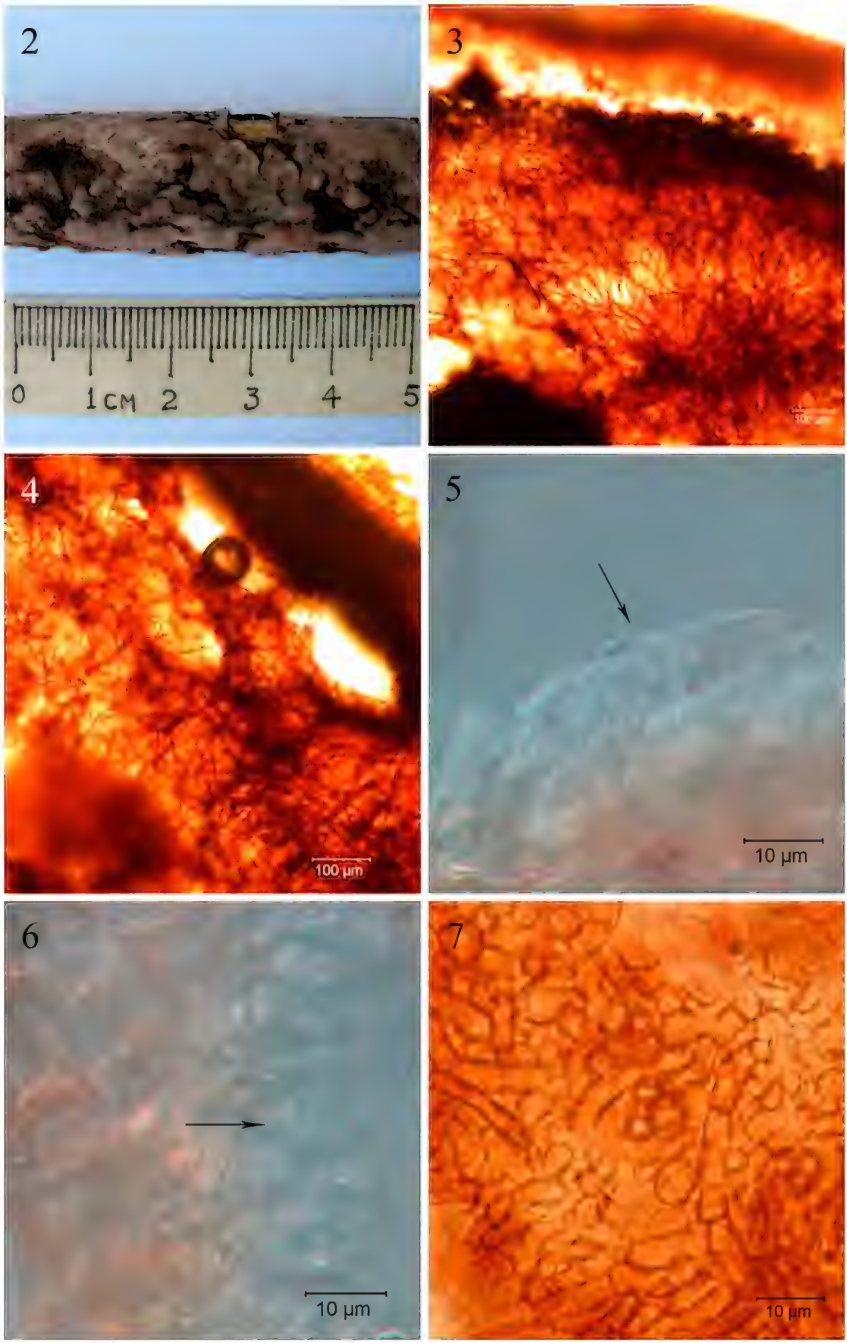


Fig. 1. Basidia and basidiospores of *Septobasidium saurauiae* (HMAS 263145, holotype).

Basidiomata on branches, resupinate, growing discontinuously, 6–7 cm long, 3–4 cm wide, cinnamon-brown or grey-brown; margin determinate, surface smooth, frequently with round protuberance. In section 900–1330 μm thick. Subiculum brown, 30–50 μm thick. Forming hyphal layer or short pillars. Pillars brown, 20–50 μm high, 20–70 μm wide. Hyphal layer 780–970 μm high. From hymenial layer the fungal hyphae often renew growth to form the second hyphal layer and hymenium. Second hyphal layer 200–240 μm high. Hymenium hyaline, 40–50 μm thick. Basidia arising directly from the hyphae without a probasidial cell, at first obovoid, later cylindrical, straight or slightly curved, 4-celled, 42–50 × 6–8 μm, hyaline. Basidiospores 10–25 × 4–6 μm, hyaline. Haustoria hyaline, consisting of irregularly coiled hyphae.

Remarks: Morphologically, *Septobasidium saurauiae* is similar to *S. meizhouense* C.X. Lu et al., which differs in numerous cracks in the basidioma surface and a thinner section (400–650 μm high) and hyphal layer (220–440 μm high) (Lu et al. 2010).

Figs. 2–7 (right). *Septobasidium saurauiae* (HMAS 263145, holotype). 2. Basidiomata on branch. 3–4. Sections of basidiomata. 5–6. Basidia (arrows). 7. Haustoria.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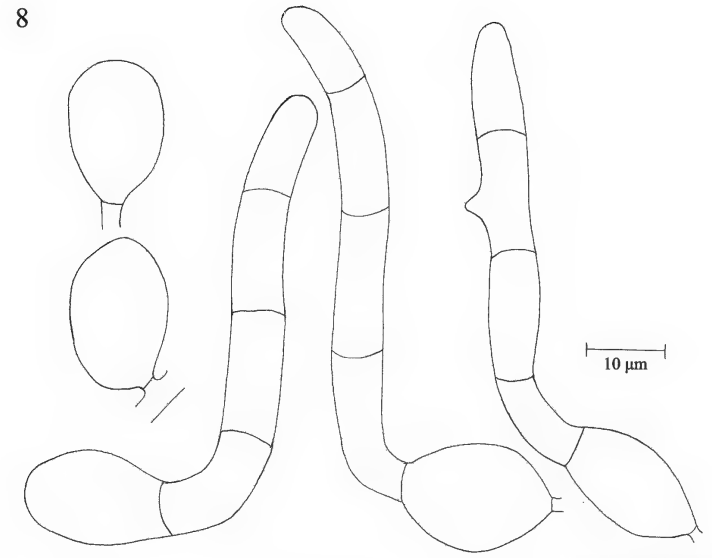


Fig. 8. Basidia and probasidia of *Septobasidium pseudopedicellatum* (HMAS 242745).

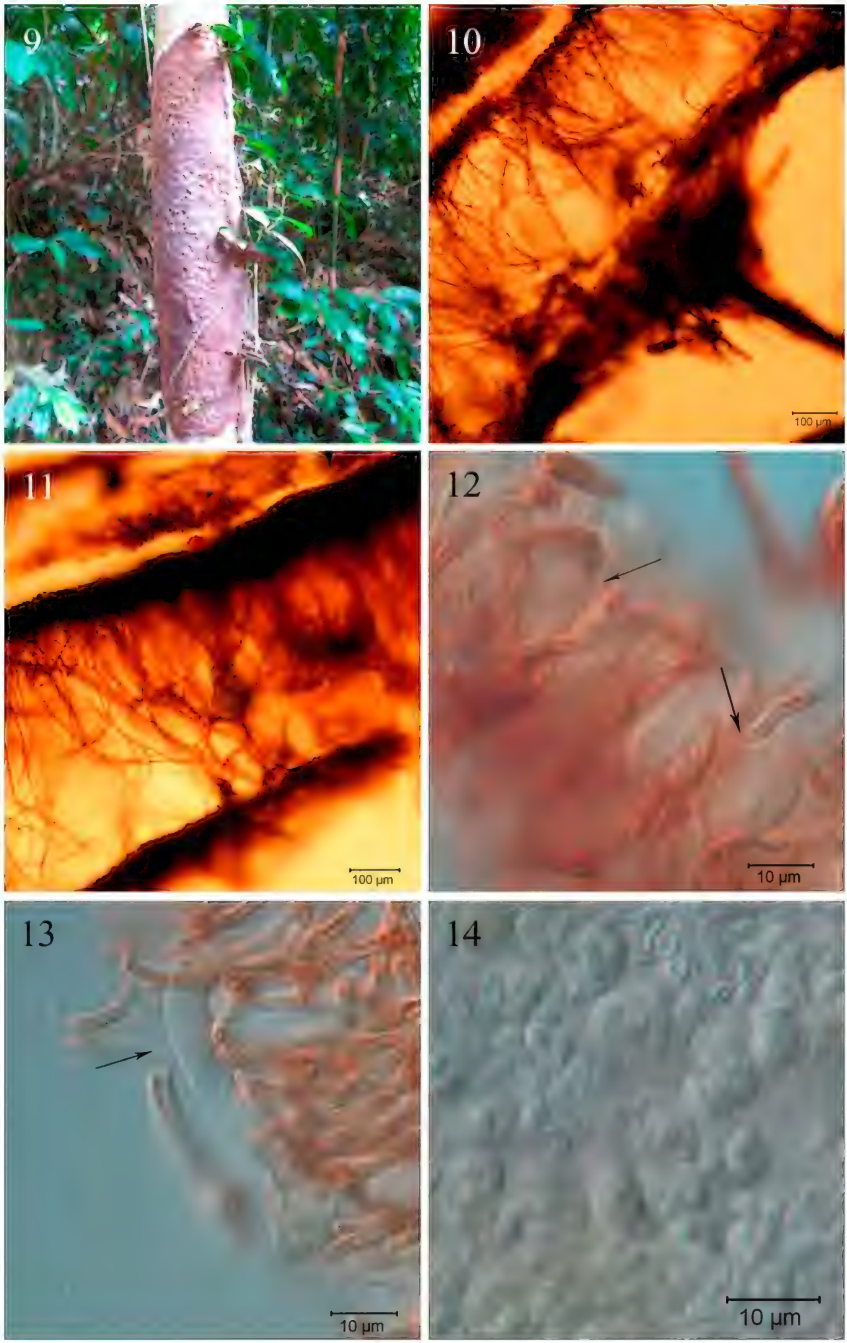
Septobasidium pseudopedicellatum Burt, Ann. Mo. bot. Gdn 3: 327, 1916.

Figs. 8–14

Basidiomata on trunks, branches and leaves, resupinate, 0.3–19 cm long, 0.2–9 cm wide, chestnut brown, brown or smoke grey; margin white, determinate, surface smooth. The pillars near the margin are visible with naked eyes. In section 850–1700 μm thick. Subiculum white or brown, 30–50 μm thick. Pillars brown, 500–700 μm high, 50–170 μm wide. The pillars branch out to form hyphal layer, 400–650 μm high. From hymenial layer the fungal hyphae often renew growth to form the second hyphal layer and hymenium, up to 3-strata. Hymenium brown, 40–60 μm thick, with closely packed paraphyses that are parallel, upright and curved at top. Probasidia hyaline or brownish, obovoid or ellipsoidal, 16–20 × 10–16 μm. Basidia cylindrical, straight or slightly curved, 4-celled, 52–62 × 5–7.5 μm, hyaline, with persisting probasidial cell. Sterigmata conical, 2.5–8 × 2.5–3.5 μm. Basidiospores not seen. Haustoria consisting of irregularly coiled hyphae.

Specimens examined: **CHINA**, Hainan Province, Bawangling Natural Reserve, Nanchahe, alt. 600 m, on *Schefflera octophylla* (Lour.) Harms (*Araliaceae*), 14.IV.2011, L.Guo 11611, HMAS 242745; on *Psychotria serpens* L. (*Rubiaceae*), 14.IV.2011, L.Guo 11612, HMAS 251216.

Figs. 9–14 (right). *Septobasidium pseudopedicellatum* (HMAS 242745). 9. Basidiomata on trunk. 10–11. Sections of basidiomata. 12. Probasidia (arrows). 13. Basidium (arrow). 14. Haustoria.


9
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100 µm
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100 µm
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13
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10 µm

Including the two species reported in this paper, 35 *Septobasidium* species have now been reported in China (Sawada 1933, Couch 1938, Teng 1963, Tai 1979, Kirschner & Chen 2007, Lu & Guo 2009a,b,c, 2010a,b,c, 2011, Lu et al. 2010, Chen & Guo 2011a,b).

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Hallenbergia (*Agaricomycetes*), a new corticioid genus

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Abstract – A new corticioid genus, *Hallenbergia*, and its single species, *H. singularis*, are described from Thimphu, Bhutan.

Key words – Eastern Himalaya, Nawephu, angiosperm host

While conducting a fungal foray in Nawephu of Thimphu, Bhutan, Dhingra made a collection on decaying angiospermous twigs. On the basis of macroscopic and microscopic characters it was compared with similar genera within *Corticiaceae* s.l. (Rattan 1977, Eriksson & Ryvarden 1976, Hjortstam et al. 1987) but could not be assigned to any already known, hence the description of a new genus. Morphological traits show similarities with *Hypochnicium* and *Intextomyces*.

Hallenbergia Dhingra & Priyanka, **gen. nov.**

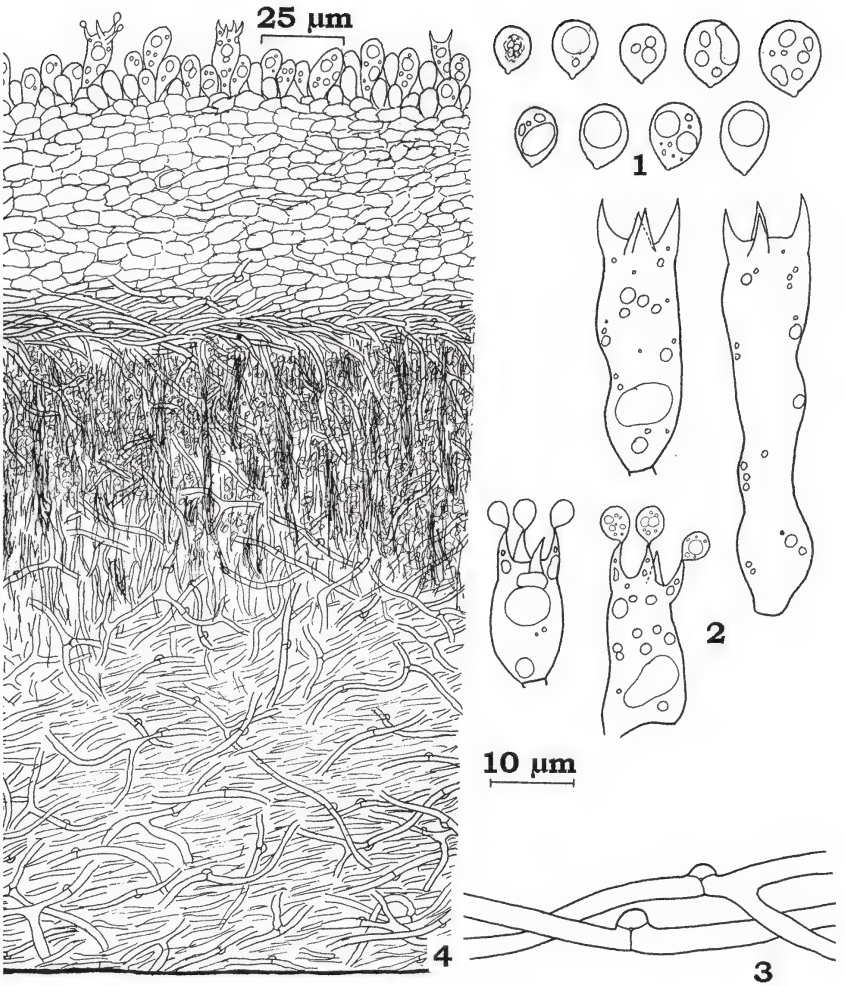
MycoBank MB 560467

Basidiocarpum resupinatum, adnatum, effusum, ceraceum; hymenium laevigatum, farinaceum, subvitrum, continuum, rimosum in sicco; systema hyphale monomiticum; hyphae tenuitunicatae, septatae, fibulatae; hyphae basilarae irregulariter ramosae, dense intertextae; hyphae in subhymenio parvae-loculosae, compaginatae et apparenter cellulosae; cystidia absum; basidia subclavatae ad suburniformes, 4-sterigmatae; basidiosporae ellipsoidae ad ovoidae vel globosae, laeves, crassitunicatae, cyanophilae, inamyloidae.

Type Species: *Hallenbergia singularis* Dhingra & Priyanka

Etymology: The name of the genus is in the honour of Prof. Nils Hallenberg.

Basidiocarp resupinate, adnate, effused, thin, ceraceous; hymenial surface smooth, farinose under the lens, continuous, some cracks developing on drying; margins not well differentiated. Hyphal system monomitic; generative hyphae thin-walled, septate, clamped; basal hyphae irregularly branched and interwoven into a dense texture; subhymenial hyphae short-celled, compactly packed and appear like a cellular tissue. Cystidia absent. Basidia subclavate to



Figs 1–4. *Hallenbergia singularis*: microscopic structures:
1. basidiospores; 2. basidia; 3. generative hyphae; 4. vertical section through basidiocarp.

suburniform, 4-sterigmate. Basidiospores ellipsoid to ovoid or subglobose, smooth, with thickened walls, cyanophilous, inamyloid.

Remarks—*Hallenbergia* resembles the genus *Hypochnicium* in having broadly ellipsoid to subglobose basidiospores with somewhat thick and cyanophilous walls. There are also affinities with *Intextomyces* in having a densely interwoven texture. The new genus differs from both genera by the peculiar hyphal texture,

Fig. 5. *Hallenbergia singularis*: basidiocarp showing hymenial surface.

with a densely and irregularly branched lower part and a pseudoparenchymatous upper part. Moreover, it differs from *Intextomyces* in the way basidia are formed. In *I. contiguus* (P. Karst.) J. Erikss. & Ryvarden, basidia are formed at the apex of penetrating, sinuous hyphae, while in *H. singularis* the basidia are directly produced from the surface of the pseudoparenchymatic tissue. A sample has been studied by Prof. John Eriksson and Prof. Nils Hallenberg, who both supported the concept of a new genus.

Hallenbergia singularis Dhingra & Priyanka, **sp. nov.** Figs 1–5

MycoBank MB 560468

Basidiocarpum resupinatum, adnatum, effusum, ceraceum, ad 250 µm crassum; hymenium laevigatum, farinaceum, subvitrum, continuum, rimosum in sicco; systema hyphale monomiticum; hyphae ad 4.5 µm latae, tenuitunicatae, fibulatae; hyphae basilariae irregulariter ramosae, intricatae in textura compacta; hyphae in subhymenio parvae-loculosae, compaginatae et appariter cellulosae; cystidia absum; basidia 15–33 × 7–10.5 µm, subclavatae ad suburniformes, 4-sterigmatae; basidiosporae 6–9 × 4.5–7.5 µm, latae ellipsoidae ad ovoidae vel globosae, laeve, crassitunicatae, cyanophilae, inamyloidae, un-i vel multiguttatae.

Type: Bhutan, Thimphu, Nawephu, on decaying angiospermous twigs, 31 July 1981, Dhingra 19548 (PAN, **holotype**).

Etymology: The epithet refers to the uncommon and strange combination of microscopic features.

Basidiocarp resupinate, adnate, effused, thin, up to 250 µm thick in section, ceraceous; hymenial surface smooth to farinose under the lens, yellowish gray to orange gray, continuous when fresh, some cracks developing on drying;

margins not well-differentiated. Hyphal system monomitic; generative hyphae up to 4.5 µm wide, thin-walled, septate, clamped; basal hyphae irregularly branched and interwoven into a dense texture, covered in the upper half by some crystalline material, followed by a narrow zone of compactly packed horizontal hyphae; subhymenial hyphae short-celled, compactly packed and appear like a cellular tissue. Cystidia absent. Basidia 15–33 × 7–10.5 µm, at first ellipsoid, then broadly subclavate to suburniform, rarely sinuous, basal clamp not observed, 4-sterigmate, with oily contents; sterigmata up to 7 µm long; a new basidium generally takes the place of a decomposed basidium. Basidiospores 6–9 × 4.5–7.5 µm, broadly ellipsoid to ovate or subglobose, smooth, somewhat thick-walled, cyanophilous, inamyloid, with one large guttule or many small oil drops.

Remarks—As mentioned above *Hallenbergia singularis* is superficially similar to *Intextomyces contiguus*. The latter species is easily distinguished by smaller spores and basidia.

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Cylindrochytridium johnstonii is a member of the *Cladochytriales*

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Abstract — The taxonomy of the *Chytridiomycota* has been in flux between a classical system based on thallus morphology and a new system based on zoosporic ultrastructure and analyses of genetic sequences. *Chytridiales* sensu Sparrow has been divided into 7 orders plus undescribed lineages. We found and brought into pure culture *Cylindrochytridium johnstonii*, the type species of the genus, which heretofore has not been characterized by molecular methods. We confirmed that this species is a member of the *Cladochytriales*, but it does not lie within a recognized family.

Key words — nucLSU rDNA, nucSSU rDNA

Introduction

Molecular studies have led to new orders being segregated from the large chytridiomycete order *Chytridiales* sensu Sparrow (Barr 1980; Letcher et al. 2006, 2008; Longcore & Simmons 2012; Mozley-Standridge et al. 2009; Simmons et al. 2009), yet many genera and species were not represented in these studies because they were unavailable in culture. Such species, which were classified in the *Chytridiales* in the classical literature (e.g., Sparrow 1960, Karling 1977), remain incertae sedis in the *Chytridiomycetes*. Therefore, when chytrids that have not been studied with molecular tools are isolated into pure culture it is appropriate to determine their taxonomic position in the new system and provide photographs of developmental morphology. Reporting genetic data for described species is intended to promote universal taxon concepts. The phylum *Chytridiomycota* has previously relied on thallus morphologies and zoosporic ultrastructure, both of which, at least to some extent, have led to paraphyletic groupings (e.g. *Chytridiales* sensu Sparrow 1960, *Chytridiales* sensu Barr 1980) compared to molecular phylogenies (James et al. 2000, 2006).

The genus *Cylindrochytridium* was described by Karling (1941) for *C. johnstonii*, a species with unique developmental morphology that occurred on boiled grass used as bait in samples of aquatic debris. Subsequently, *C. johnstonii* has been

reported from sites around the world, including New Zealand (Karling 1968), South America (Marano et al. 2008a,b; Rocha & Pires-Zottarelli 2002), Europe (Czeczuga et al. 2007), and India (Karling 1963), but no authors have reported isolating this fungus into pure culture. Its phylogenetic position therefore has remained unconfirmed. We found this species growing on the edge of onionskin bait placed with algae and aquatic debris collected from Smith's Fen (Schwintzer 1978) during a field trip that was part of the 2008 centennial celebration of the University of Michigan Biological Station in northern Michigan, USA. Herein we report that *C. johnstonii* is a member of the *Cladochytriales* and support this conclusion with molecular data. Also, we include photos of the development of this chytrid in pure culture for comparison with the type description.

Materials & methods

Collection, isolation & morphology

JE Longcore collected algae and plant debris on 22 Sep 2008 from Smith's Fen (pH = 5.7; Schwintzer 1978), University of Michigan Biological Station, Cheboygan County, Michigan, USA. The sample was transported at ambient temperature in a Whirlpak® plastic bag to our laboratory in Maine. We placed the aquatic sample in a finger bowl at room temperature and baited it with pieces of boiled, white onionskin. After several weeks we noted thalli with catenulated rhizoids on the bait. Most of the thalli grew at the edges of the onionskin and produced long, cylindrical zoosporangia. We rinsed the onionskin with a stream of distilled water and placed it in a depression slide with distilled water. After ~1 hour, when many active zoospores could be seen, we added the water to plates of TC agar (0.4 g tryptone, 4 g cellobiose, 10 g agar, 1 L distilled water) containing 300 mg/L streptomycin and 200 mg/L penicillin G. We monitored the isolation plates at 100× magnification by inverting them on the stage of a compound microscope. After nearly two weeks, we selected *Cylindrochytridium johnstonii*, recognized by its long, cylindrical zoosporangia, from among the other chytrids on the isolation plate and removed thalli to clean plates of TC agar. We transferred the isolate, designated JEL596, to mPmTG slants (Longcore 1992) in screw-topped 125 × 20 mm culture tubes and maintained the stock tubes at room temperature. Stocks were transferred at 3-month intervals. To document morphology and days to maturity, we inoculated cultures on mPmTG agar in 9 cm Petri plates stored at 23 °C. To document morphology on cellulosic substrates, we inoculated a flask of sterilized lake water containing pieces of white onionskin with thalli near maturity or with motile zoospores present and incubated the flasks at room temperature. We aseptically removed portions of agar with thalli from plates and pieces of onionskin from flasks to view the chytrid by light microscopy. We photographed developmental stages with phase contrast and Hoffman Modulation Contrast (HMC) optics on a Nikon E400 microscope (Nikon Instruments, Melville, New York) equipped with a Spot RT digital camera (Diagnostic Instruments, Sterling Heights, Michigan). Composite images of thalli through multiple focus planes were assembled from overlapping micrographs of individuals in Adobe Photoshop 6.0 (Adobe Systems Inc., San Jose, California).

TABLE 1. Isolates of *Cladochytriales* used for phylogenetic analyses of *Cylindrochytridium johnstonii*

ISOLATE	CULTURE NO.	ORIGIN	HABITAT	GENBANK ACCESSION NO.	
				nucSSU	nucLSU
INGROUP:					
Allochytridium expandens	Barr253	North Carolina, USA	Soil/detritus	AF164291S1	EU828501
Allochytridium luteum	Barr463	Ontario, CAN	Sandy soil/detritus	AY349047	AY349082
Catenochytridium sp. 1	JEL145	Maine, USA	Aquatic/*Eriocaulon*	EU828475	EU828503
Catenochytridium sp. 2	JEL024	Maine, USA	Aquatic/detritus	EU828476	EU828504
Catenochytridium sp. 3	JEL044	Maine, USA	Aquatic/detritus	EU828478	EU828506
Cladochytrium sp.	JEL153	Maine, USA	Aquatic/maple leaf	EU828458	EU828485
Cylindrochytridium johnstonii	JEL596	Michigan, USA	Aquatic/detritus	JF796051	JF796052
Diplophlyctis sp.	JEL331	Maine, USA	Aquatic/*Characeae*	EU828477	EU828505
Endochytrium ramosum	JEL402	Michigan, USA	Aquatic/*Cladophora*	EU828484	EU828513
Endochytrium sp. 1	JEL027	Maine, USA	Aquatic/*Eriocaulon*	EU828471	EU828498
Endochytrium sp. 2	JEL070	Maine, USA	Aquatic/*Eriocaulon*	EU828472	EU828499
Endochytrium sp. 3	JEL324	Maine, USA	Aquatic/*Elodea*	EU828473	EU828500
Nephrochytrium aurantium	JEL036	Maine, USA	Aquatic/*Sparganium*	EU828468	EU828495
Nephrochytrium sp. 1	JEL327	Maine, USA	Aquatic/*Nitella*	EU828467	EU828494
Nephrochytrium sp. 2	JEL125	Maine, USA	Aquatic/*Characeae*	AF164295S1	EU828511
Nowakowskiella elegans	JEL046	Maine, USA	Aquatic/detritus	EU828463	EU828490
Nowakowskiella elegans	JEL127	Maine, USA	Aquatic/*Characeae*	EU828466	EU828493
Septochytrium sp.	JEL177	Wales, UK	Aquatic/detritus	EU828474	EU828502
Septochytrium variabile	JEL191	Ontario, CAN	Aquatic/*Eriocaulon*	AH009045	EU828512
Unidentified sp.	JEL072	Maine, USA	Aquatic/*Eriocaulon*	EU828470	EU828497
OUTGROUP					
Karlingiomyces sp.	JEL093	Maine, USA	Aquatic	AF164278	AY349085
Polychytrium aggregatum	JEL109	Maine, USA	Aquatic	AY601711	AY349084

Sequencing & analyses

DNA of JEL596 was extracted with Whatman® FTA card technology (Whatman Ltd., Maidstone, Kent, UK; Borman et al. 2006, Simmons 2011). Segments of nucSSU and nucLSU rDNA were amplified, sequenced, and aligned as in Simmons (2011) with taxa from *Cladochytriales* (Table 1) after a BLAST search in GenBank grouped JEL596 within that order. A region of nucSSU rDNA was amplified with the NS1/NS4 primer pair (White et al. 1990), as was a region of nucLSU rDNA with the LR0R/LR5 primer pair (Rehner & Samuels 1994, Vilgalys & Hester 1990). The Akaike Information Criterion in jModeltest 0.1.1 (Guindon & Gascuel 2003, Posada 2008) chose the TIM3+G model of evolution for the combined dataset. We entered these parameters into MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003), which ran for 1M generations, selecting every 100th tree. A 50% majority rule phylogram was constructed and MP bootstrap and BPP values were computed in PAUP* 4.0b10 (Swofford 2002) as in Simmons (2011).

Taxonomy

Cylindrochytridium johnstonii Karling, Bull. Torrey Bot. Club 68: 383 (1941)
Figure 1

Type: Karling (1941: Figs. 1–16).

Specimen examined. **USA. Michigan.** On onionskin substrate added to water culture of algae and aquatic plant detritus, 22 Sep 2008. JEL596 (nucSSU rDNA sequence GenBank JF796051; nucLSU rDNA sequence GenBank JF796052).

We emend the description of *Cylindrochytridium johnstonii* by adding morphology on agar and axenic onionskin and molecular sequence information for JEL596.

Thallus characters — In pure culture, isolate JEL596 has morphological characters similar to Karling's (1941, 1977) descriptions of *C. johnstonii*. On agar, germlings developed endogenously, forming catenulate swellings at a single axis or multiple rhizoidal axes (Fig. 1A, B). The catenulations and the spherical base of what would become the zoosporangium developed relatively

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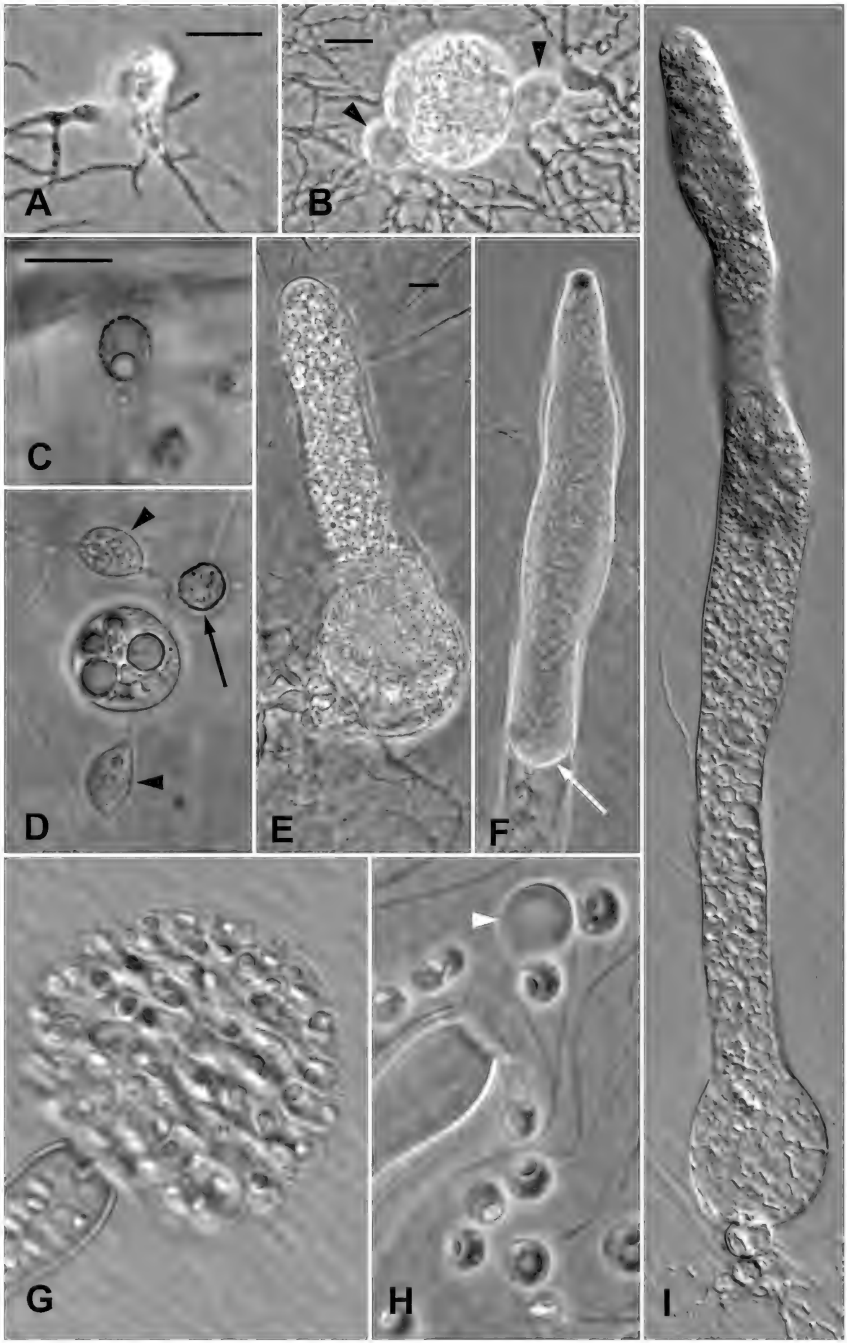
Figure 1 (right). *Cylindrochytridium johnstonii* on mPmTG agar and liquid medium grown at 23 °C and on onion skin grown at room temperature. Micrographs taken with phase contrast optics unless otherwise noted. A. Germling at 3 days with catenulated rhizoids. B. Developing thallus at 8 days with two catenulated rhizoidal axes (arrowheads) and diffusely branching rhizoids. C. Brightfield micrograph of amber, ovoid zoospore cyst on onion skin with ridges along surface of cell wall and a single, large lipid globule. D. Brightfield composite micrograph of exogenously developing germling in onion skin, with zoospore cyst (arrow) and catenulated rhizoids (arrowheads). E. Composite micrograph of broad tube extending from spherical base of zoosporangium. F. Septum (white arrow) with tapered nipple delimiting zoosporangium base and apically migrating cytoplasm in thallus. G. Hoffman Modulation Contrast (HMC) micrograph of zoospores released from apical pore. H. HMC micrograph of spherical zoospores after release from zoosporangium by dehiscence of operculum (white arrowhead). I. Composite HMC micrograph of developing thallus with catenulated rhizoids, vacuolated portion of zoosporangium towards spherical base, and apically migrating cytoplasm. Scale bars = 10 μm; bar in A for G–H; bar in C for D; bar in E for F,I.


A
B
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slowly as the rhizoidal system expanded through the agar (Fig. 1B). After nearly two weeks, one side of the spherical zoosporangium elongated to form a tube nearly as wide as the spherical base (Fig. 1E). Most of the cytoplasm migrated toward the apical portion of the broad tube, leaving a highly vacuolated portion at the zoosporangium base (Fig. 1F, I). At maturity, the basal portion of the zoosporangium was empty and may have been walled off by a septum (Fig. 1F). Zoospores exited the zoosporangium through a pore made by the complete detachment of an apical operculum (Fig. 1H). Zoospores possessed a single lipid globule (Fig. 1G, H), and flagella were ~35 μm long.

Our attempts to reintroduce *C. johnstonii* to onionskin led to the production of germlings, but after 1 month, no further development occurred. On onionskin isolate JEL596 developed exogenously. The amber, ovoid zoospore cyst on the surface of the onionskin had a cell wall with ridges and generally possessed a single, large lipid globule (Fig. 1C). Germlings possessed one or two rhizoidal axes with catenulations (Fig. 1D).

Phylogenetics — A majority rule Bayesian phylogram (Fig. 2) was constructed from a data matrix with 337 parsimony-informative characters of a total matrix of 1410 characters. The phylogeny indicates that *Cylindrochytridium johnstonii* is within the *Cladochytriales*. The species is in a clade that is sister to a clade containing the type species of *Allochytridium* Salkin (Salkin 1970) and *Septochytrium* Berdan (Berdan 1939), which are the only two genera recognized in *Septochytriaceae* (Mozley-Standridge et al. 2009).

Discussion

Our phylogeny (Fig. 2) places *Cylindrochytridium johnstonii* as sister to an isolate with *Catenochytridium* morphology (JEL145). Together these two taxa are sister to a group of isolates in the *Septochytriaceae* (Mozley-Standridge et al. 2009) and are also part of a larger clade (Fig. 2), whose members form large catenulations in their rhizoids. One species in this clade, *Allochytridium expandens* Salkin, also has an elongated zoosporangium, but not to the extent seen in *C. johnstonii*. Additionally distinguishing these two taxa, *A. expandens* develops exogenously both in natural substrates and on agar medium (Barr 1986) whereas *C. johnstonii* develops endogenously on agar medium.

Shanor (1944) commented upon the resting spore of *C. johnstonii*, but he did not illustrate this feature. He described the resting spore on filter paper as being light-brown or amber, nearly spherical, with a smooth, thick cell wall and one large yellow oil globule. This description is very similar to that of the zoospore cyst that we saw on onionskin, but fine ridges appeared on the surface of most zoospore cysts we observed. Though the amber zoospore cysts on onionskin may look like resting spores, their connection to larger portions of the thallus (Fig. 1D) lead us to conclude they are zoospore cysts. Thus, we believe Shanor may have mistaken the zoospore cyst for a resting spore.

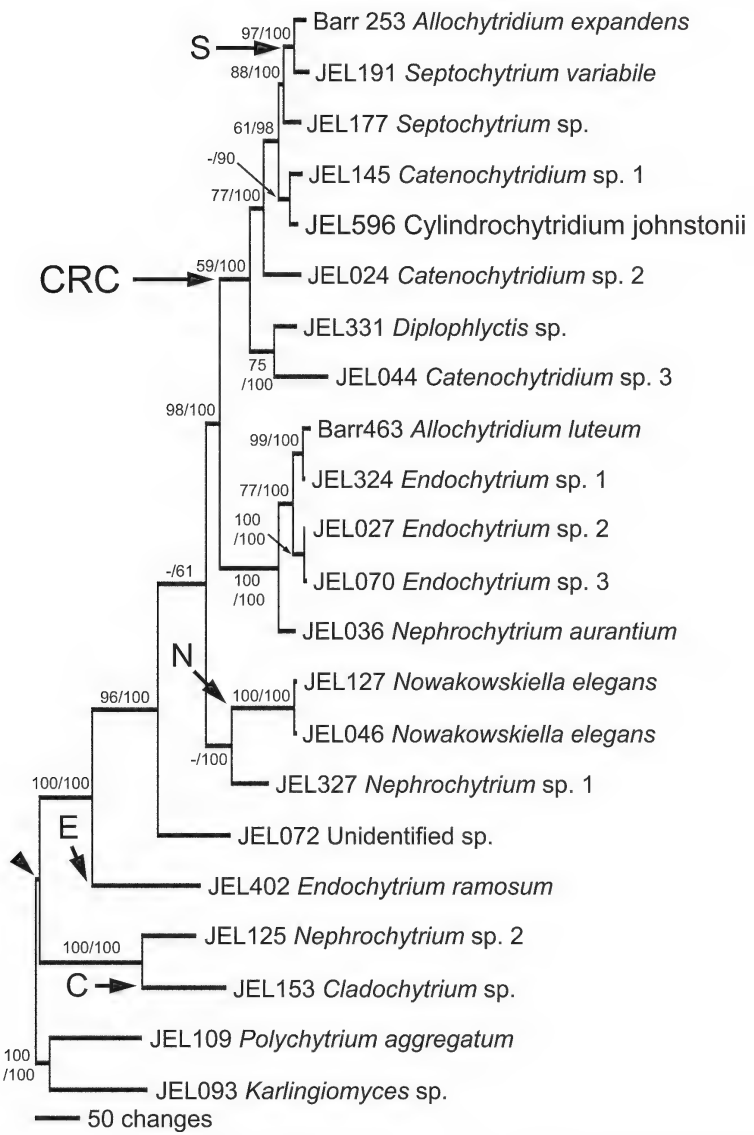

FIGURE 2. Majority rule consensus Bayesian phylogram of combined nucSSU and nucLSU rDNA for 20 taxa of *Cladochytriales* and 2 isolates in the *Polychytrium* clade (James et al. 2006). Labeled nodes correspond to the catenulated rhizoids clade (CRC) and families within the *Cladochytriales* (arrowhead): *Septochytriaceae* (S), *Nowakowskiellaceae* (N), *Endochytriaceae* (E), and *Cladochytriaceae* (C) (Mozley-Standridge et al. 2009). Branch support values are listed as maximum parsimony bootstrap values / Bayesian posterior probabilities. Tree length = 1234, CI = 0.6183, RI = 0.6235.

Karling (1941) illustrated elongation of *C. johnstonii* thalli as beginning during growth of the germling, with some zoosporangia having basal swellings. The developing zoosporangia we examined on agar were spherical (Fig. 1B) and grew an apical elongation only when nearly mature (Fig. 1E, F, I). Additionally, young thalli of our isolate on onionskin were also spherical (Fig. 1D).

Considering that Karling (1941) observed *C. johnstonii* in gross culture and we studied our isolate in pure culture, our observations agree well with his drawings, which constitute the type of *C. johnstonii*. *Cylindrochytridium endobioticum* Willoughby (Willoughby 1964) is the only other species in the genus, but Karling (1977) considered it to be a dubious member because of its considerable variation from *C. johnstonii*. Our genetic information provides the basis for adding additional species to the genus as well as evaluating the suitability of the genus for *C. endobioticum*, when it is found and brought into culture.

Acknowledgments

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A contribution to the study of smut fungi of Israel

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Abstract — Four species of smut fungi, *Antherospora vaillantii, Microbotryum holostei, Urocystis magica,* and *U. muscaridis,* are reported for the first time in Israel. *Urocystis magica* was found in the Judean desert on a new host plant, *Allium rothii,* and *M. holostei* is new for Asia.

Key words — *Urocystales*, biodiversity, *Microbotryales*, Middle East

Introduction

The smut fungi that occur in Israel have been studied by mycologists and phytopathologists since the start of the 1900s (Magnus 1900; Reichert 1921, 1930, 1931; Săvulescu & Rayss 1935; Rayss & Zwirn 1944; Rayss 1952; Palti et al. 1966). Nevertheless, knowledge about species composition and distribution of these fungi was incomplete until a critical revision of the published accounts of smut fungi in Israel that showed 59 species were recorded (Savchenko et al. 2010).

During February–March 2011, we collected plants infected by smut fungi, mainly in the territory of Mount Carmel, a coastal mountain range in northern Israel that stretches southeast from the Mediterranean Sea. This region is botanically very rich in species with a typical East-Mediterranean flora with luxuriant herbaceous components. Some of them are host plants for smut fungi. In the present study we report three smut species found in this region and one species from the Judean Desert.

Materials & methods

Sorus and spore characteristics were studied using both fresh and dried herbarium specimens. Spores were dispersed in a droplet of lactophenol on a microscope slide,

covered with a cover glass, gently heated to boiling point to rehydrate the spores, cooled, and then examined by a Carl Zeiss Axiostar light microscope (LM) at 1000× magnification. For scanning electron microscopy (SEM), spores were attached to specimen holders by double-sided adhesive tape and coated with gold. The surface structure of spores was observed at 15 kV and photographed with a scanning electron microscope JEOL JSM-6700F. Specimens used in this study are stored in the Herbarium of Haifa University (HAI).

Results & discussion

Descriptions and critical notes of four new Israeli species of smut fungi are given below.

Antherospora vaillantii (Tul. & C. Tul.) R. Bauer et al., Mycol. Res. 112: 1304 (2008) FIGS. 1–2

SORI in the anthers and on the surface of inner floral organs, producing dark olive-brown powdery mass of spores, enclosed by the floral petals. Infection systemic, all flowers of an inflorescence are infected. SPORES variable in shape and size, globose, subglobose, ovoid, slightly elongated, 7–8.5 × 8–10(–11) μm, olive-brown. SPORE WALL even, ca. 0.5–0.7 μm thick, finely, densely verruculose. In SEM spores densely irregularly verruculose. SPORE PROFILE appears wavy.

DISTRIBUTION: Europe, Asia, Africa, North America, Oceania.

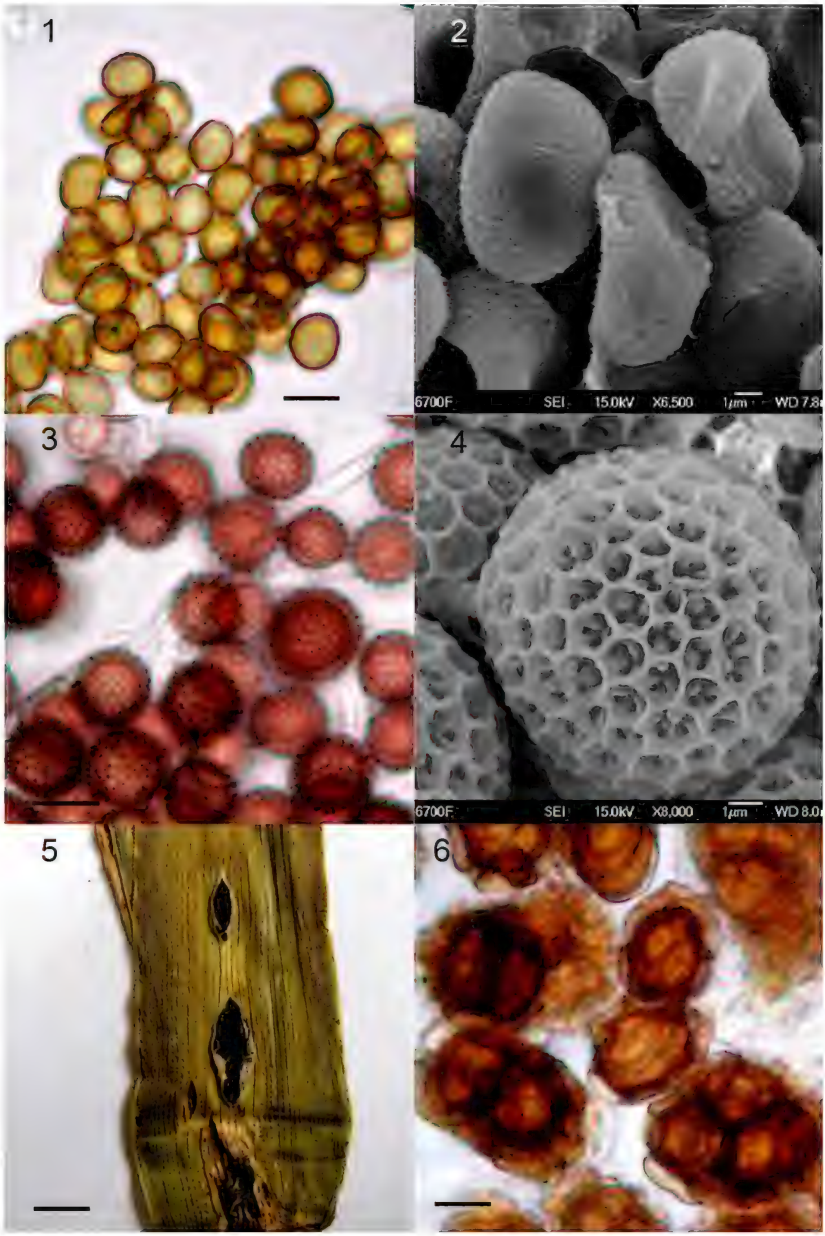
SPECIMEN EXAMINED: **ISRAEL. HAIFA DISTRICT**, Carmel National Park, 32°75′79″N, 35°01′50″E, on *Muscari comosum* (L.) Mill., 2.II.2011, leg. K.G. Savchenko (HAI 2857).

NOTE. The genus *Antherospora* R. Bauer et al. consists of eight species of smut fungi that infect the anthers and inner floral organs of host plants in the *Hyacinthaceae* (*Liliaceae* s.l.) (Vánky 2009). The genus differs from all other taxa of *Urocystales* by the organogenic specialization, lack of sterile cells, and development of single spores (Bauer et al. 2008). Most of its species are highly specialized parasite restricted to certain host plant genera. Only one other *Antherospora* species, *A. urgineae* (Maire) R. Bauer et al. on *Urginea maritima* (L.) Baker, is known to occur in Israel (Săvulescu & Rayss 1935, Savchenko et al. 2010). *Antherospora vaillantii* is easily distinguishable from *A. urgineae*, which has smaller spores (9.5–15(–17.5) × 7–12 μm in diam.) and hosts in different genera.

Microbotryum holostei (de Bary) Vánky, Mycotaxon 67: 44 (1998) FIGS. 3–4

SORI in ovules, fill the capsules with a powdery reddish brown spore mass. Infection systemic. SPORES reddish-brown, globose, sometimes ovoid, 9–14 × 11–14 μm. SPORE WALL reticulate, 4–6 meshes per spore diam., meshes 1.2–2.4 μm in diam.; the bottom of the meshes with conspicuous round low protuberances.

DISTRIBUTION: Europe, Asia.



Figs. 1–6. **1–2**: spores of *Antherospora vaillantii* in flowers of *Muscari comosum*. **3–4**: spores of *Microbotryum holostei* in flowers of *Holosteum umbellatum*. **5–6**: sori and spores of *Urocystis muscaridis* on leaves of *Muscari comosum*. 1, 3, 6 = LM; 2, 4 = SEM. Scale bars: 1, 3, 6 = 10 μm; 2, 4 = 1 μm; 5 = 3 mm.

Specimen examined: **ISRAEL. Haifa district**, Carmel National Park, 34°74′97″N, 35°03′01″E, on *Holosteum umbellatum* L. (*Caryophyllaceae*), 3.II.2011, leg. K.G. Savchenko (HAI 2858).

Note. Only three species of *Microbotryum* Lév. were reported from Israel before our investigations: *M. cordae* (Liro) G. Deml & Prillinger on *Polygonum acuminatum* Kunth (*Polygonaceae*), *M. jehudanum* (Zundel) Vánky on *Silene apetala* Willd. (*Caryophyllaceae*), and *M. scorzonerae* (Alb. & Schwein.) G. Deml & Prillinger on *Scorzonera papposa* DC. (*Asteraceae*) (Rayss 1952, Rayss & Zwirn 1944, Savchenko et al. 2010). All three are typical ovaricolous species. Israel has a great diversity of potential hosts for smuts from *Microbotryum*, especially those parasitizing *Caryophyllaceae* (43 *Silene* species grow in the country; Feinbrun-Dothan & Danin 1998). *Microbotryum holostei* has probably been overlooked because of the inconspicuous symptoms of infection and prevernal development. To our knowledge, *M. holostei* has never been reported from Asia.

Urocystis magica Pass., in Thümen, Mycoth. Univ. 3: no. 223 (1875) Figs. 7–9

Sori as pustules in leaves and bulbs, initially covered by an epidermis that later ruptures to expose the black mass of spore balls. Spore balls globose to ovoid, 18–25 × 20–40 µm in diam., composed of 1 (90%) to 2 (10%) central spores and continuous layer of peripheral sterile cells. Spores globose, subglobose, ovoid, 10–15 × 12–16 µm in diam., dark reddish-brown. Spore wall ca. 1 µm thick. Sterile cells globose, ovoid, irregular, 4–13 µm in diam., pale yellowish-brown.

Distribution: Europe, Asia, North America, South America, Africa, Oceania.

Specimen examined: **ISRAEL. South district**, Judean Desert, near Arad, Wadi Kidot, 31°15′73″N, 35°13′59″E, alt. 569 m., on *Allium rothii* Zucc. (*Alliaceae*), 15.III.2011, leg. K.G. Savchenko (HAI 2860).

Note. During a collection trip to the Judean Desert in March 2011, one *Allium rothii* plant was found infected by a smut fungus. *Allium rothii* belongs to the subgenus *Melanocrommyum* and is distributed in Israel in the Judean Desert, North and South Negev, and the Dead Sea area on rocky slopes between shrubs (Kamenetsky 1994). Microscopic examination revealed that the smut was *U. magica* s.l., not previously recorded in Israel (Savchenko et al. 2010). *Allium rothii* represents a new host plant for this fungus.

Allium L. is a major genus of the monocot family *Alliaceae* naturally distributed throughout the northern hemisphere that is represented by ca. 750 species worldwide (Stearn 1992). Almost 40 species are known in Israel (Feinbrun-Dothan & Danin 1998). According to recent taxonomical changes based on nrDNA ITS sequence analyses, *Allium* is divided into 15 well-delimited monophyletic subgenera (Friesen et al. 2006). Species from

Figs. 7–9. *Urocystis magica* on *Allium rothii*. 7: infected plant; 8: sori: 9: spore balls (in LM). Scale bars: 7 = 1 cm; 8 = 3 mm; 9 = 10 µm.

six subgenera —*Allium, Amerallium, Cepa, Melanocrommyum, Polyprason, Rhizirideum*— have been found as hosts for *Urocystis* Rabenh. ex Fuckel. At least five different *Urocystis* taxa have been described from onion plants during the last two centuries (see Vánky 1994): *U. magica*, *U. cepulae* Frost, *U. colchici* f. *allii-subhirsuti* Beltrani, *U. allii* Schellenb., and *Tuburcinia oblonga* Massenot (≡ *U. oblonga* (Massenot) H. Zogg). As these taxa are not easily distinguished from one another, they have been treated as a single taxon, *U. magica* s.l. However, these taxa were described from different subgenera of *Allium*: *U. magica* s.str. from subg. *Melanocrommyum*, *U. cepulae* from subg. *Cepa*, *U. colchici* f. *allii-subhirsuti* from subg. *Amerallium*, and *U. allii* and *T. oblonga* from subg. *Allium*. Specialization on certain host plant subgenera or even species is not unusual among smut fungi (cf. *Entyloma* species on *Eryngium* L.; Vánky 2009). Thus, it is not inconceivable that these *Urocystis* taxa may be recognized as "good" or at least "cryptic" species after intensive molecular-phylogenetic research.

Urocystis muscaridis (Niessl) Moesz, Kárpát-mend. Üszög.: 199 (1950) FIGS. 5–6

SORI in leaves as ellipsoidal pustules, variable in size, visible on the outer surface along the viens, filled by a black powdery mass of spore balls. SPORE BALLS globose, subglobose, ovoid to irregular, 18–40 × 20–45 µm in diam., composed of 1–7(–8) spores surrounded by almost a continuous layer of sterile cells. SPORES globose, ovoid to irregular, 9–17(–18) × 10–20(–21) µm in diam., dark reddish-brown. SPORE SURFACE smooth. STERILE CELLS variable in shape and size, globose, ovoid to irregular, 4–9 × 5–14 µm, yellowish-brown.

DISTRIBUTION: EUROPE, Asia.

SPECIMEN EXAMINED: **ISRAEL. HAIFA DISTRICT**, Carmel National Park, 32°75′25″N, 35°02′50″E, on *Muscari comosum* (L.) Mill., 18.III.2011, leg. K.G. Savchenko (HAI 2862).

NOTE. *Urocystis muscaridis* infects different species of *Muscari* Mill. in Europe and Asia (Vánky 1994). *Muscari comosum*, a principal host for this smut, is quite common in Mediterranean forests and semi-steppe shrub lands of Israel. In March 2011 we examined a large *M. comosum* population in Carmel National Park and found one plant with leaves bearing several swollen sori produced by *U. muscaridis*. It is interesting that only a single plant in the whole population was found to be affected. In the Middle East *U. muscaridis* is also known from Iran (Ershad & Deghani 2001).

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Species of *Rhytismataceae* on *Lithocarpus* spp. from Mt Huangshan, China

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Abstract—Seven members of the *Rhytismataceae* are reported from leaves of tanoaks from Mt Huangshan, China. Among them *Coccomyces mucronatoides* and *Terriera coacervata* are new species, *Terriera illiciicola* is a new combination, and the other species are already known for China. This paper provides descriptions, discussions for these species as well as a dichotomous key for the eight rhytismataceous species known to occur on tanoaks worldwide.

Key words—*Rhytismatales*, morphology, taxonomy, *Fagaceae*

Introduction

Worldwide, at least 55 genera (+ 30 synonyms) and 728 species of *Rhytismataceae* have been reported (Kirk et al. 2008). These fungi are parasites on leaves, stems, twigs, or fruits of gymnosperms, angiosperms and pteridophytes or saprobes on plant residues. More than 240 different fungi have been discovered on *Lithocarpus* spp. (*Fagaceae*), but records of the *Rhytismataceae* are few (Farr & Rossman 2011). *Coccomyces dentatus* (J.C. Schmidt & Kunze) Sacc. on *Lithocarpus densiflorus* (Hook. & Arn.) Rehd. is known from the USA (Sherwood 1980), and five *Rhytismataceae* species —*C. delta*, *C. huangshanensis*, *C. mucronatus*, *Lophodermium agathidis*, *L. illiciicola*— have been reported on *Lithocarpus* spp. from China (Lin et al. 2000a,b, 2001, 2005; Wang et al. 2006).

Mt Huangshan in southern Anhui Province is suitable for many rhytismataceous species because of the warm climate, abundant rainfall, and high plant diversity. Seven species representing three different genera on *Lithocarpus* leaves are described from Mt Huangshan in this paper, including two new species, one new combination, and four species already known in China.

Materials & methods

Leaves with bleached spots were collected from plants on Mt Huangshan, and those with open fruit bodies characteristic of *Rhytismataceae* were selected. Observations of the external shape, size, color, opening mechanisms of ascomata and conidiomata, and zone line characteristics were made using a stereoscope with 10–50× magnification.

Microscopic preparations were made in water, Melzer's reagent, 5% KOH, or 0.1% (w/v) cotton blue in lactic acid. For observing the outlines of ascomata and conidiomata, vertical sections were mounted in lactic acid or cotton blue with water pretreatment. Gelatinous sheaths surrounding ascospores and paraphyses were observed in water or cotton blue in lactic acid. The color of structures and ascospore contents were observed in water. Measurements and drawings of asci, ascospores, and paraphyses were made from material mounted in 5% KOH or Melzer's reagent from 30 ascospores, asci and paraphyses for each specimen. Point and line integrated illustrations of external shapes and internal structures of the fruit bodies were drawn using a Panasoianic XSJ-2 microscopic painting device.

Taxonomy

Coccomyces delta (Kunze) Sacc., Boletím da Sociedade Broteriana 11: 13, 1893
≡ *Phacidium delta* Kunze, Linnaea 5: 551, 1830

Type: **MADEIRA**, on leaves of *Lauraceae*. [Without further data.]

Zone lines frequent, black, thin, surrounding the bleached spots.

Conidiomata not observed.

Ascomata on both sides of leaves, scattered in irregular, yellow-brown bleached spots. In surface view, ascomata (700–)1000–1600(–1800) µm diam., triangular or quadrangular, black, shiny, slightly raising the substratum surface, opening by 3–4 radial splits. Lips present. In median vertical section, ascomata intraepidermal. Covering stroma up to 25–28 µm thick near the opening, black to dark-brown, becoming thinner or firstly thickening then becoming thinner towards the edge, composed of textura globulosa or angularis with cells 4–7 µm diam. Lip cells fringed, 1.5–2.2 µm diam., immersed in a gel. Basal stroma slightly hollow or flat, 4.0–6.5 µm thick, dark-brown, composed of textura globulosa or angularis with cells, connecting to the covering stroma. Internal matrix stroma only between the covering stroma and basal stroma. Subhymenium 15–20 µm thick, consisting of textura intricata and porrecta. Paraphyses 100–130 × 1.5–2.0 µm, filiform, aseptate, gradually swollen to 2.2–3.0 µm above, covered in a thin mucous coating. Asci ripening sequentially, 85–120 × 7.5–9.5(–11) µm, cylindric-clavate or subclavate, somewhat long-stalked, apex thin-walled, J–, 8-spored. Ascospores fasciculate, 52–85 × 1.5–1.8(–2.0) µm, filiform, hyaline, aseptate, with a ca 1 µm thick gelatinous sheath.

Illustration: Sherwood (1980: Fig. 18).

Ecology & distribution: Ascomata found on fallen or not yet fallen diseased leaves. Widely distributed in Europe (Sherwood 1980). In China, it is known from southern parts (Lin et al. 2000a).

Specimens examined: On *Lithocarpus glaber* (Thunb.) Nakai: **China**, **Anhui**, Mt Huangshan, alt. ca 550 m, 11 August 1995, S.M. Yu, Y.R. Lin 1596a (AAUF 67794a); Ciguangge, alt. ca 800 m, 4 October 2009, Q. Zheng, S.J. Wang 5436 (AAUF 71544).

Comments—The species was originally described from Madeira, and mainly occurs on plants of the *Lauraceae* and evergreen *Quercus* (Sherwood 1980). Teng (1934) first reported *C. delta* from China. According to our observation, it infects living leaves at early stage, and may be followed by other *Coccomyces* species as the ascomata mature. Compared with the polygonal species in the genus, *C. delta* ascomata are the largest. The asci and ascospores in the Mt Huangshan specimens are shorter than described by Sherwood (1980).

Coccomyces huangshanensis Y.R. Lin & Z.Z. Li, Mycosystema 19: 449, 2000

Type: **China**, **Anhui**, Mt. Huangshan, Songgu'an, alt. ca 600 m, on *Cyclobalanopsis glauca* (Thunb.) Oerst., 9 August 1995, Y.R. Lin & S.M. Yu 1632 (AAUF 67740).

Zone lines frequent, black-brown, thin, surrounding the bleached areas.

Conidiomata with a distribution on the leaf similar to that of the ascomata. In surface view, conidiomata 180–330 × 150–260 μm, subcircular or elliptical, brown in the centre and at the perimeter line of the conidioma, concolorous with the substratum surface or light grey-brown over the rest of the appearance, slightly raising the surface of the leaf. In vertical section, conidiomata intraepidermal, double lens-shaped. Upper wall very thin, brown to dark-brown, with an indefinite structure. Basal wall 4–7 μm thick, brown, composed of thick-walled, angular cells. Conidiogenous cells 5.5–9.0 × 1.5–2.0 μm, flask-shape, holoblastic sympodially proliferating. Conidia 3.5–5.0 × ca 0.8 μm, bacilliform, hyaline, aseptate.

Ascomata on both sides of leaves, more on the upper side of the leaf, scattered in irregular bleached spots. In surface view, ascomata 700–1100 μm diam., triangular or quadrangular, black-brown, slightly raising the substratum surface, opening by 3–4 radial splits. Lips present at least in the early stages. In median vertical section, ascomata intraepidermal. Covering stroma 15–18 μm thick near the opening, becoming thinner towards the edge, extending to the basal stroma, consisting of brown to dark-brown textura angularis with cells 3.5–6.5 μm diam. The opening often covered by the heavily carbonized tissue. Lip cells tend to disappear as the ascomata mature. Periphysoids present. Basal stroma 8–12 μm thick, dark-brown, consisting of textura angularis or globulosa with cells 5.5–8 μm diam. Internal matrix stroma only between covering stroma and basal stroma. Excipulum 22–30 μm thick, arising from the inner layer of the covering stroma. Subhymenium 10–18 μm thick,

consisting of textura porrecta. Paraphyses 110–135 × 1.5–2.0 μm, filiform, septate, slightly swollen at the apex. Asci ripening sequentially, 85–115 × 6.0–7.0 μm, cylindrical, short-stalked, apex obtuse, thin-walled, J–, 8-spored. Ascospores fasciculate, 60–90 × 1.2–1.5 μm, filiform, hyaline, aseptate, with a thin gelatinous sheath, sometimes with a gelatinous cap at the top.

Illustration: Lin et al. (2000b: Fig. 2).

Ecology & distribution: Ascomata found on decaying leaves. Known only from China (Lin et al. 2000b).

Specimens examined: On *Lithocarpus henryi* (Seemen) Rehder & E.H. Wilson: **China**, **Anhui**, Mt Huangshan, Tangkou, alt. ca 500 m, 29 September 1993, Y.R. Lin, L. Chen 1511a (AAUF 67619a); 2 October 2009, Q. Zheng, X.M. Gao 5389 (AAUF 71497).

Comments—*Coccomyces limitatus* (Berk. & M.A. Curtis) Sacc. is very similar to this species but differs in the well developed internal matrix of the stroma, 50 μm thick excipulum, and dissimilar asci and ascospores. Its asci are 4.0–5.6 μm wide, and the ascospores are only 0.8–1.0 μm wide (Sherwood 1980; Johnston 1986).

Coccomyces mucronatus Korf & W.Y. Zhuang, Mycotaxon 22: 487, 1985

Type: **China**, **Sichuan**, Mt Qingcheng, alt. ca 900 m, on *Castanopsis* sp., 6 July 1983, W.Y. Zhuang HMAS 450558 (CUP-CH 2484).

Zone lines frequent, black-brown to black, thin, surrounding or separating the bleached spots.

Conidiomata not observed.

Ascomata on both sides of leaves, scattered in irregular, grey-yellow bleached spots. In surface view, ascomata 650–1200 μm diam., polygonal to subcircular, black-brown to black, slightly raising the substratum surface, opening by radial splits. Lips absent. In median vertical section, ascomata intraepidermal to subepidermal. Covering stroma 15–22 μm thick, consisting of grey-brown to dark-brown textura angularis and globulosa with cells 3.5–7 μm diam., extending to the basal stroma. Periphysoids, 8–15 × 2.0–3.0 μm, cylindrical, hyaline, 0–3 septate. Basal stroma 8–12 μm thick, composed of dark-brown textura angularis and globulose. Internal matrix stroma poorly developed, 10–20 μm thick in the center, hyaline, consisting of gelatinous textura intricata. Excipulum 40–70 μm thick in the upper, abruptly thinner towards the base, arising from the inner layer of the covering stroma. Subhymenium 15–20 μm thick, composed of colourless textura porrecta or angularis. Paraphyses 110–140 × 2.0–2.5 μm, filiform, abruptly enlarged to 4.5–5.5 μm and subfusoid-ventricose above, with a 2.5–6.0 × 1.0–2.0 μm, subcylindrical mucro at the apex, often agglutinated. Asci ripening sequentially, 104–119 × 4.2–5.0 μm, cylindrical, short-stalked, apex round to obtuse, thin-walled, J–, 8-spored. Ascospores fasciculate, 60–86 × 0.6–0.8 μm, filiform, hyaline, aseptate, with an indefinite gelatinous sheath.

Illustration: Korf & Zhuang (1985: Fig. 2).

Ecology & distribution: Ascomata found on decaying leaves. Widely distributed in Indonesia (Korf & Zhuang 1985). In China, it is known from Sichuan and Anhui provinces (Lin et al. 2001).

Specimens examined: On *Lithocarpus glaber*: **China**, **Anhui**, Mt Huangshan, Tangkou, alt. ca 550 m, 11 August 1995, Y.R. Lin, L. Chen 1596b (AAUF 67704b). On *L. henryi*: **China**, **Anhui**, Mt Huangshan, Tangkou, alt. ca 550m, 29 September 1993, Y.R. Lin et al. 1511b (AAUF 67619b); Taoyuanting, alt. ca 650 m, 10 June 1994, Y.R. Lin, S.M. Yu 1569a (AAUF 67677a); Wenquan, alt. ca 700 m, 3 October 2009, Q. Zheng, X.M. Gao 5411 (AAUF 71519).

Comments— Korf & Zhuang (1985) first described *C. mucronatus* based on specimens on fallen leaves of *Castanopsis* sp. from Sichuan in China and Java in Indonesia. The Mt Huangshan specimens usually produce fruit bodies on decaying fallen leaves or leaf fragments, suggesting that the species is not implicated in any plant disease. The other features of this species agree with those of the type specimen described by Korf & Zhuang (1985), except for the inlaid depth of the ascomata and the growth pattern of the excipulum.

Coccomyces mucronatoides Y.R. Lin, Q. Zheng & S.M. Yu, **sp. nov.** Figs. 1–6

MycoBank MB561883

Ascomata (480–)600–1100 µm, trigona usque ad hexagona, flavo-brunnea, subepidermalia. Periphysoidei rarissimi. Excipulum 10–15µm crassum, ex hyphis hyalinis septatis parallelis constatum. Paraphyses filiformes, apice abrupte tumidae. Asci 85–120 × 4.5–5.0µm, cylindrico-clavati. Ascosporae 70–105 × 0.8–1.0 µm, filiformes, hyalinae, vagina gelatinosa tenui indutae.

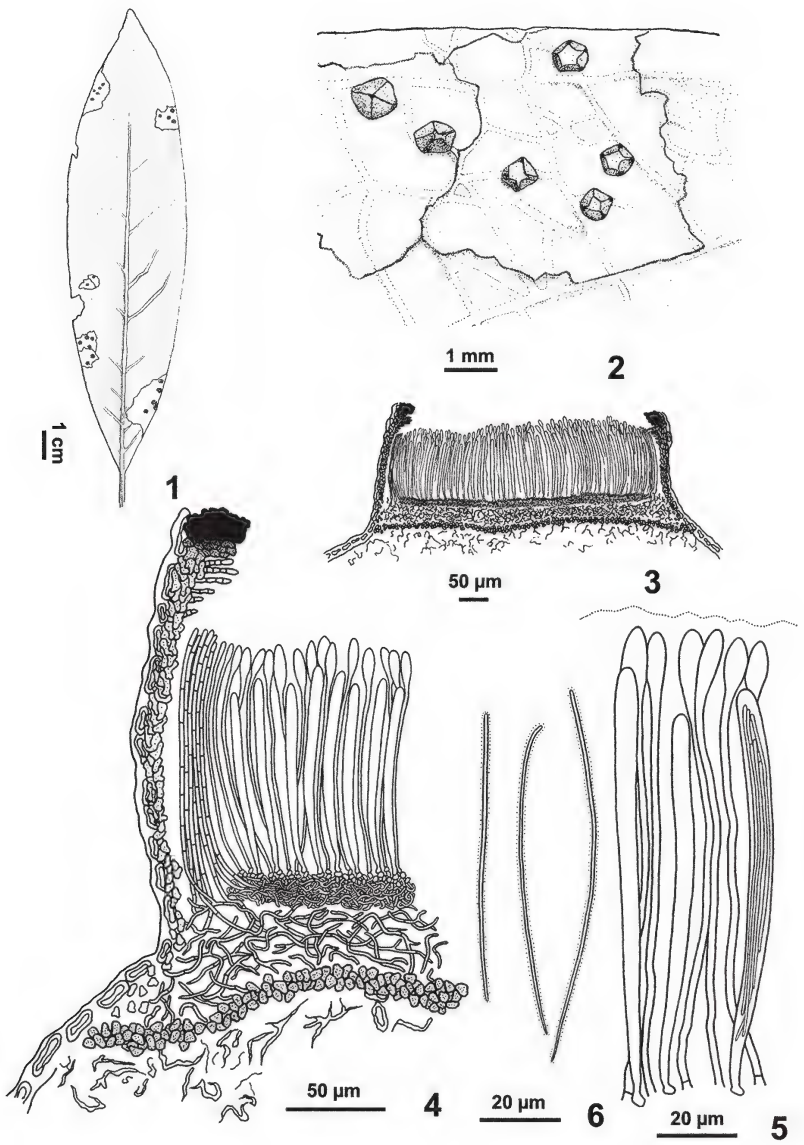
Type: China, Anhui, Mt Huangshan, Tangkou, alt. ca 550 m, on *Lithocarpus cleistocarpus* (Seemen) Rehder & E.H. Wilson, 11 September 2007, S.J. Wang, Y.R. Lin 2244b (**Holotype**, AAUF 68352b).

Etymology: referring to the similarity of the paraphysis apex to *Coccomyces mucronatus* in shape.

Zone lines somewhat frequent, black-brown or yellow-brown, thin, surrounding the bleached spots.

Conidiomata not observed.

Ascomata developing on both sides of leaves, more on the upper side of the leaf, scattered in irregular, yellowish-white or yellowish beached spots. In surface view, ascomata (480–)600–1100 µm diam., triangular to hexagonal, yellow-brown, not shiny, with a clearly defined edge, somewhat raising the substratum surface and flattened or slightly concave in the central region, with an obvious preformed dehiscence mechanism, opening by 3–6 radial splits, which extend nearly to the edge of ascoma, to expose the sallow hymenium. Lips absent. In median vertical section, ascomata subepidermal. Covering stroma 25–32 µm thick near the opening and 8–15 µm thick in other parts, composed of an upper part of textura globulosa with black-brown cells 5–6 µm



Figs. 1–6. *Coccomyces mucronatoides* on *Lithocarpus cleistocarpus* (from holotype). 1. A leaf bearing ascomata. 2. Ascomata as seen under a dissecting microscope. 3. Ascoma in median vertical section. 4. Detail of ascoma in median vertical section. 5. Paraphyses and asci. 6. Discharged ascospores.

diam., an lower part of textura epidermoidea with dark-brown cells 3.0–4.5 µm diam., and a few yellowish angular cells almost connecting to the basal stroma. Periphysoids extremely sparse, (5–)8–15 × 2.0–2.5 µm, cylindrical, hyaline, (1–)3–5 septate. Basal stroma moderately developed, 10–15(–18) µm thick, composed of black-brown textura angularis or globulosa with thick-walled cells 5–7 µm diam. Internal matrix stroma well developed, 20–30(–45) µm thick, consisting of hyaline, gelatinised textura intricata. Excipulum 10–15 µm wide, composed of rows of hyaline, septate, thin-walled, parallel hyphae. Subhymenium 15–22 µm thick, composed of hyaline textura intricata and angularis. Paraphyses 100–140 × 1.8–2.0 µm, filiform, aseptate, abruptly enlarged to 4.0–6.0 µm and elliptical or subfusoid near the apex, cemented in a thin gel, forming an epithecium 15–20 µm thick. Asci ripening sequentially, 85–120 × 4.5–5.0 µm, cylindrical, stalked, apex round to obtuse, thin-walled, J–, 8-spored. Ascospores arranged in a fascicle, 70–105 × 0.8–1.0 µm, filiform, hyaline, aseptate, with a thin gelatinous sheath.

Ecology & distribution: Ascomata found on dead leaves in litter. Known only from the type locality.

Comments—According to the type descriptions, *C. mucronatus* differs from the new species in frequent periphysoids, poorly developed internal matrix that occurs only between covering stroma and basal stroma, and paraphyses with an abruptly swollen subfusoid-ventricose upper part with a mucro at the tip (Korf & Zhuang 1985). *Coccomyces mucronatus* on *Lithocarpus* spp. observed by the authors differs from *C. mucronatoides* in an excipulum arising from the inner layers of covering stroma, with 1–2 dark-brown, swollen cells at the apex. However, the ascomata of the new species from Mt Huangshan have few periphysoids, a well-developed internal matrix, and non-mucronate paraphyses with a suddenly enlarged elliptical or subfusoid body near the apex.

Lophodermium agathidis Minter & Hettige, New Zealand Journal of Botany 21: 39, 1983

Type: **NEW ZEALAND**, **Auckland**, Waitakere Ranges, Oratia, Kelly's Bush, on *Agathis australis* (D. Don) Lindl. ex Loudon, 10 November 1979, G. Hettige 259066 (IMI).

Zone lines usually frequent, surrounding the bleached spots.

Conidiomata with a distribution on the leaf similar to that of the ascomata, scattered to crowded. In surface view, conidiomata 140–300 µm diam., somewhat round, black-brown in the centre and at the perimeter line of the conidioma, concolorous with the substratum surface elsewhere, or grey-brown to black-brown as a whole. In vertical section, conidiomata subepidermal. Upper wall 4–7 µm thick, dark-brown, consisting of thick-walled angular cells 2–4 diam. Basal wall brown to dark-brown, composed of 1–2 layers of

thin-walled angular cells 3–5 µm diam. Subconidiogenous layer 7–10 µm thick, consisting of thick-walled angular cells. Conidiogenous cells 10–16 × 2.0–3.0 µm, flask-shape, proliferating sympodially. Conidia 3.0–4.0 × ca 1.0 µm, elliptical, hyaline, aseptate.

Ascomata on both sides of leaves scattered in irregular, yellow-brown bleached spots. In surface view, ascomata 800–1900(–2300) × 390–650 µm, elliptical to elongate-elliptical, black, slightly raising the substratum surface, opening by a longitudinal split. Lips light reddish-brown. In median vertical section, ascomata subepidermal. Covering stroma consisting of thick-walled aliform-angular cells 3–7 µm diam., 55–70 µm thick and heavily carbonized near the opening, other parts dark-brown, becoming suddenly thinner towards the edge. Lip cells arranged in a subparallel manner or slightly radially, cylindrical. Basal stroma 6–10 µm thick, dark-brown, consisting of thick-walled aliform-angular cells 4–7 µm diam. Subhymenium ca 12 µm thick, hyaline, composed of angular cells. Paraphyses 110–160 × 1.5–2.0 µm, filiform, irregularly swollen to 2.0–3.0 µm or occasionally branched above, covered with a mucous coating. Asci maturing sequentially, 100–145 × 6–7.5 µm, cylindrical, short-stalked, apex round, thin-walled, J–, 8-spored. Ascospores arranged in fascicles sometimes helically coiled, 70–95 × 1.2–1.6 µm, filiform, hyaline, 0–1-septate, enveloped in a ca 0.5 µm thick gelatinous sheath.

Illustration: Minter & Hettige (1983: Figs. 1–9).

Ecology & distribution: Ascomata found on dead leaves in litter. Widely distributed in China (Lin et al. 2005), Malaysia (Spooner 1991) and New Zealand (Johnston 2001).

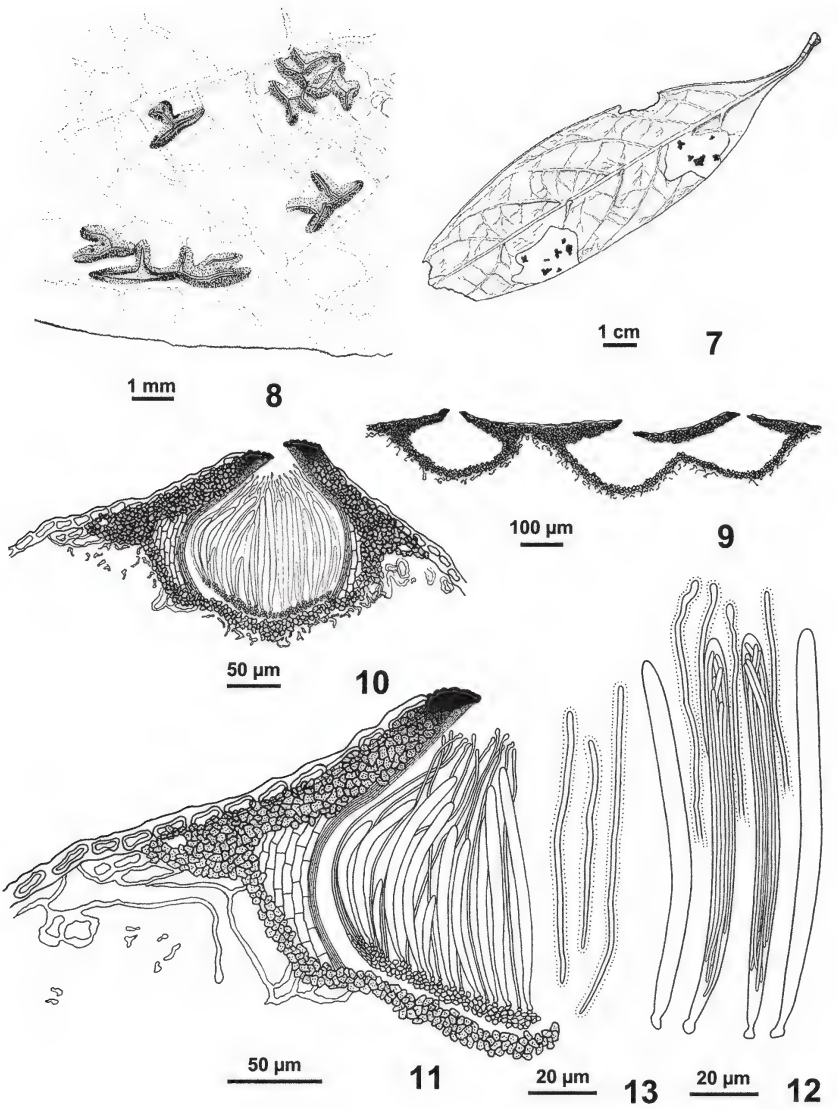
Specimens examined: On *Lithocarpus glaber*: **China**, **Anhui**, Mt Huangshan, Tangkou, alt. ca 550 m, 5 April 1994, Y.R. Lin et al. 1655 (AAUF 67763). On *L. henryi*: **China**, **Anhui**, Mt Huangshan, Tangkou, alt. ca 550 m, 2 October 2009, Q. Zheng, X.M. Gao 5385 (AAUF 71493).

Comments—*Lophodermium agathidis* occurs on a wide range of gymnosperms and angiosperms (Minter & Hettige 1983; Johnston 1989a, 2001). Minter & Hettige (1983) inferred that it is probably a leaf endobiont inhabiting apparently healthy leaves that fruits only after death of the leaf from some other cause. After examination of specimens from China, Lin et al. (2005) found that on leaves of different plants, *L. agathidis* differs in thickness of the covering stroma, location of the lip cells, and size of asci and ascospores.

Terriera coacervata Y.R. Lin & Q. Zheng, **sp. nov.** Figs. 7–13

MycoBank MB561884

Ascomata coalescentia. Singula ascomata (650–)1000–1800(–2200) × 350–560 µm, plerumque elliptica, subepidermalia. Extentio valde carbonacea ad apicem stromatis tegentis affixa. Excipulum maxime debiliter evolutum. Paraphyses filiformes. Asci 90–130



Figs. 7–13. *Terriera coacervata* on *Lithocarpus cleistocarpus* (from holotype). 7. A leaf bearing ascomata. 8. Ascomata as seen under a dissecting microscope. 9. Three coalescent ascomata in median vertical section. 10. Ascoma in median vertical section. 11. Detail of ascoma in median vertical section. 12. Paraphyses and asci. 13. Discharged ascospores.

× 6.0–7.0 µm, cylindrici. Ascosporae 60–110 × 1.5–1.8 µm, filiformes, hyalinae, vagina gelatinosa indutae.

Type: China, Anhui, Mt Huangshan, Tangkou, alt. ca 550 m, on leaves of *Lithocarpus cleistocarpus*, 11 September 2007, S.J. Wang, Y.R. Lin 2244a (**Holotype**, AAUF 68352a).

Etymology: referring to the coalescent form of the ascomata.

Zone lines infrequent, thin, grey-brown to brown, sometimes not defined, surrounding or partly surrounding the bleached spots.

Conidiomata absent.

Ascomata developing on lower side of leaves, 2–9 ascomata coalescent in irregular, yellowish bleached spots 15–25 µm diam. In surface view, ascomata interlaced or accumulatively coalescent, forming multilocular fruit bodies. Single ascomata (650–)1000–1800(–2200) × 350–560 µm, elliptical, sometimes branching into lobed or polygonal shapes, black, slightly shiny, markedly raising the substratum surface, opening by a longitudinal split more than 4/5 the length of the ascoma or by not less than 3 lobes. Lips absent. In median vertical section, ascomata subepidermal with epidermal cells becoming filled with fungal tissue as ascomata develop. Covering stroma 20–30 µm thick, composed of black-brown textura angularis-globulosa with thick-walled cells. Along the edge of the ascoma opening, there is a short extension, about 12 µm thick, adjacent to the covering stroma, which is comprised of strongly carbonized tissue with no obvious cellular structure. Basal stroma strongly concave, 10–18 µm thick, composed of 3–4 layers of black-brown, angular and globose cells 2.8–4.0 µm diam., extending to the covering stroma. Colorless to light grey-brown textura prismatica 10–22 µm thick exists between the covering stroma and basal stroma. Excipulum very poorly developed, arising from the inner layer of the basal stroma, consisting of hyaline textura porrecta. Subhymenium 8–12 µm thick, composed of hyaline textura angularis. Paraphyses 120–160 × 1.4–1.6 µm, filiform, usually gradually swollen to ca 2.0 µm at the apex, not branched, covered with a ca 0.8 µm thick mucous coating. Asci maturing sequentially, 90–130 × 6.0–7.0 µm, cylindrical, short-stalked, apex round, J–, 8-spored. Ascospores arranged in a fascicle or helically coiled, 60–110 × 1.5–1.8 µm, filiform, hyaline, aseptate, enveloped in a 1.0–1.5 µm thick gelatinous sheath.

Ecology & distribution: Ascomata found on dead or fallen leaves and known only from the type locality.

Comments—This new species has characteristics typical of *Terriera*. In each ascoma, there is a flattened extension comprising strongly carbonized tissue adjacent to the covering stroma along the edge of the ascomatal opening, a very poorly developed hyaline textura porrecta excipulum, and a colorless to light grey-brown textura prismatica between the covering stroma and basal stroma.

Terriera coacervata is unusual and unique in the genus because its ascomata usually interlace or accumulatively coalesce to form multilocular fruit bodies.

Lophodermium euryae Y.R. Lin & Y.F. He differs from *T. coacervata* in a different coalescent form, the oblong, elliptical or broadly elliptical smaller ascomata (290–650(–870) × 250–350 µm), smaller ascospores (45–70 × 0.8–1.2 µm), paraphyses with suddenly swollen to subglobose apices branched to form an epithecium, and the abundant conidiomata (Lin et al. 2005).

We discovered a hyperparasite — the anamorphic fungus, *Cladosporium lophodermii* Georgescu & Tutunaru — in some *T. coacervata* collections that infects ascomata to produce dark-brown hyphae and conidia in the interior. Hyperparasitism is frequent in some *Lophodermium* taxa (Minter 1981; Lin 1989).

Terriera illiciicola (S.J. Wang, Y.F. He & Y.R. Lin) Q. Zheng & Y.R. Lin, **comb. nov.**
MycoBank MB561885

≡ *Lophodermium illiciicola* S.J. Wang, Y.F. He & Y.R. Lin, Mycosystema 25: 1, 2006.

Type: **China**, **Anhui**, Mt Huangshan, Tangkou,, alt. ca 550 m, on *Illicium verum* Hook. f., 25 September 2004, Y.R. Lin et al. 1912 (AAUF 67683).

Zone lines frequent, black, thin, surrounding or partly surrounding the bleached spots.

Conidiomata absent.

Ascomata developing on the upper side of leaves, crowded in yellowish to yellow-brown bleached spots. In surface view, ascomata 300–380 × 280–330 µm, subcircular to broad-elliptical, black, shiny, with a clearly defined edge, moderately raising the surface of the substratum, opening by a longitudinal split. Lips absent. In median vertical section, ascomata subepidermal. Covering stroma 25–30 µm thick, consisting of black-brown textura angularis with cells 4.5–6.5 µm diam. Along the edge of the ascomatal opening, there is an extension, about 15 µm thick, adjacent to the covering stroma, which is comprised of strongly carbonized tissue with no obvious cellular structure and tends to disappear as the ascomata mature. Basal stroma concave and cup-shaped, 12–18 µm thick, consisting of black-brown textura angularis with 2(–3) layers of cells 5.0–7.5 µm diam., extending to the covering stroma. Colorless or light brown textura prismatica 15–20 µm thick exists between the covering and basal stromata. Subhymenium 12–15 µm thick, consisting of textura angularis-porrecta. Paraphyses 115–150 × 1.0–1.5 µm, filiform, the top not swollen or branched, covered with an inconspicuous mucous coating. Asci maturing sequentially, 90–135 × 4.0–5.0 µm, narrow-cylindrical or cylindric-clavate, long-stalked, apex round, J–, 8-spored. Ascospores fasciculate, 65–95 × ca 1.0 µm, filiform, hyaline, aseptate, enveloped in an inconspicuous gelatinous sheath.

Illustration: Wang et al. (2006: Fig. 1).

Ecology & distribution: Ascomata found on fallen leaves and known only in China (Wang et al. 2006).

Specimens examined: On *Lithocarpus cleistocarpus*: **CHINA, Anhui**, Mt Huangshan, Shibawan, alt. ca 1000 m, 22 September 2004, S.J. Wang, Y.F. He, Y.R. Lin 1806 (AAUF 67914).

Comments—This species was originally described as *Lophodermium illiciicola* (Wang et al. 2006) and is transferred here because its diagnostic characteristics agree with those of the genus *Terriera*. The species resembles *T. minor* (Tehon) P.R. Johnst. (Ortiz-García et al. 2003), which can be differentiated by oblong or elliptical larger ascomata (320–800(–1400) × 220–340 µm), a flat basal stroma, and paraphyses that branch 2–3 times at the top, sometimes forming an epithecium (Johnston 1988, 1989b; Lin et al. 2005).

Key to species of the *Rhytismataceae* on *Lithocarpus* spp. worldwide

1a. Ascomata polygonal, opening by radial splits 2
1b. Ascomata more or less elliptical or round, opening by a longitudinal split 6
2a. Lip cells present at least at the early stage 3
2b. Lip cells absent 4
3a. Ascomata 700–1100 µm diam., with periphysoids ..... *C. huangshanensis*
3b. Ascomata (700–)1000–1600(–1800) µm diam., without periphysoids .... *C. delta*
4a. Paraphyses gradually swollen at apex ..... *C. dentatus*
4b. Paraphyses suddenly swollen at apex 5
5a. Paraphyses swollen to subfusoid-ventricose above, with a subcylindrical mucro at apex ..... *C. mucronatus*
5b. Paraphyses swollen to elliptical or subfusoid above, without a mucro at apex ..... *C. mucronatoides*
6a. Top of covering stroma with a flattened, strongly carbonized extension 7
6b. Top of covering stroma without an extension ..... *L. agathidis*
7a. Ascomata interlaced or accumulatively coalescent; paraphyses gradually swollen above ..... *T. coacervata*
7b. Ascomata crowded; paraphyses not swollen above ..... *T. illiciicola*

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Aschersonia conica sp. nov. (*Clavicipitaceae*) from Hainan Province, China

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Abstract — *Aschersonia conica*, a new anamorphic species of the family *Clavicipitaceae*, is described and illustrated based on specimens collected in Hainan Province, China. The entomogenous fungus is characterized by conical, whitish to pale yellow stromata that are surrounded by a hypothallus and a wide base, wide ostiolar openings, cylindrical conidiogenous cells in a compact palisade, filiform sterile elements, and fusiform conidia.

Key words — morphology, taxonomy, new taxon

Introduction

Aschersonia Mont., a large genus in the family *Clavicipitaceae*, has been well studied, especially in America and Europe (Hywel-Jones & Evans 1993; Liu et al. 2005). Recently, new studies on *Aschersonia* and *Hypocrella* were carried out using both morphological and molecular techniques (Liu et al. 2006; Spatafora et al. 2007; Sung et al. 2007). Chaverri et al. (2008), who have provided the most extensive revision of *Aschersonia* since Petch (1921), accepted 32 species. They showed that *Aschersonia* is a form genus that shares similar anamorph morphologies with *Moelleriella* and *Hypocrella* but that both teleomorphic genera can be distinguished by their ascospore disarticulation and conidial shape and size. Mongkolsamrit et al. (2009) later published three additional species based on morphology combined with ITS and β-tubulin sequence analysis.

In China, Tzean et al. (1997) and Qiu et al. (2009, 2010) studied *Aschersonia* and its teleomorphic forms, *Hypocrella* Sacc., *Moelleriella* Bres., and *Samuelsia* P. Chaverri & K.T. Hodge. During a recent survey on insect pathogenic fungi in tropical forests in southern China, a previously unknown species of entomopathogenic *Aschersonia* was collected in the Jianfengling National Nature Reserve and is proposed here as a new species.

Materials & methods

Fungal measurements and microscopical observations followed Qiu et al. (2009, 2010). Spore range data exclude 5% of measurements from each end of the range, which are given in parentheses. Text abbreviations are as follows: CB = Cotton Blue, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios among all specimens studied, n = number of spores measured from a given number of specimens. Conidiomata were carefully dissected with a razor blade and mounted in water or lactic acid mixed with cotton blue on a slide. Special color terms follow Kornerup & Wanscher (1967). Specimen vouchers are deposited at the Mycological Herbarium, Fujian Agricultural and Forestry University (MHFAFU).

Taxonomy

Aschersonia conica Jun Z. Qiu, Y.B. Su & C.S. Weng, **sp. nov.** Figs. 1

MycoBank MB 561561

Stromata pyriformia vel conico-pulvinata, valde convexa, ex hyphis densis composita, deorsum effusa, hypothallum membranaceum ad 1.8 mm diam. 0.5 mm altum dilute luteum formantia, in statu recenti albida, superficie aliquot orificiis ut punctis magnis visibilibus praedita. Pycnidia plerumque singula, in medio stromatis immersa, 252–306 µm alta, 103–141 µm diam., elongate ampulliformia. Phialides cylindricae, ad 15 µm longae. Paraphyses praesentes, filiformes, flexuosae, ad 116 µm longae, 0.7 µm latae. Conidia fusoidea, utrinque acutata, 8.2–10.5 × 0.9–1.3 µm.

Type — China, Hainan Prov., Dongfang County, Jianfengling National Nature Reserve, alt. 1450 m, on *Aleyrodidae*, 29.X.2008, J.Z. Qiu, Y.B. Su & C.S. Weng 311 (**Holotype**, MHFAFU 20811).

Etymology — Refers to the conical stroma of this species.

Teleomorph: Unknown.

Stromata—Pyriform, conical-pulvinate, markedly convex, consisting of dense hyphae, base spreading, forming a pale yellow membranous hypothallus up to 1.8 mm diam., 0.5 mm high, whitish-coloured when fresh, several ostiolar openings as large dots visible on the surface.

Pycnidia—Usually single, embedded in the centre of the stroma, CB–, 252–306 µm high, 103–141 µm diam, elongate flask-shaped. Conidiogenous cells phialidic, cylindrical, up to 15 µm long.

Paraphyses—Present, linear, filiform, flexuous, up to 116 µm long, 0.7 µm wide.

Conidia—Fusoid, sometimes narrowly fusiform, with acute ends, 8.2–10.5 × 0.9–1.3 µm, L = 9.5 µm, W = 1.1µm, Q = 8.6–8.7 (n = 60/2).

Additional specimen examined — **China. Hainan Prov.**: Ledong County, Jianfengling National Nature Reserve, alt. 1350 m, on *Aleyrodidae*, 30.X.2008, J.Z. Qiu, Y.B. Su & C.S. Weng 358 (MHFAFU 20828).

Comments—*Aschersonia conica* is characterized by the whitish to pale yellow markedly convex conical stromata, long conidia, wide ostiolar openings, and

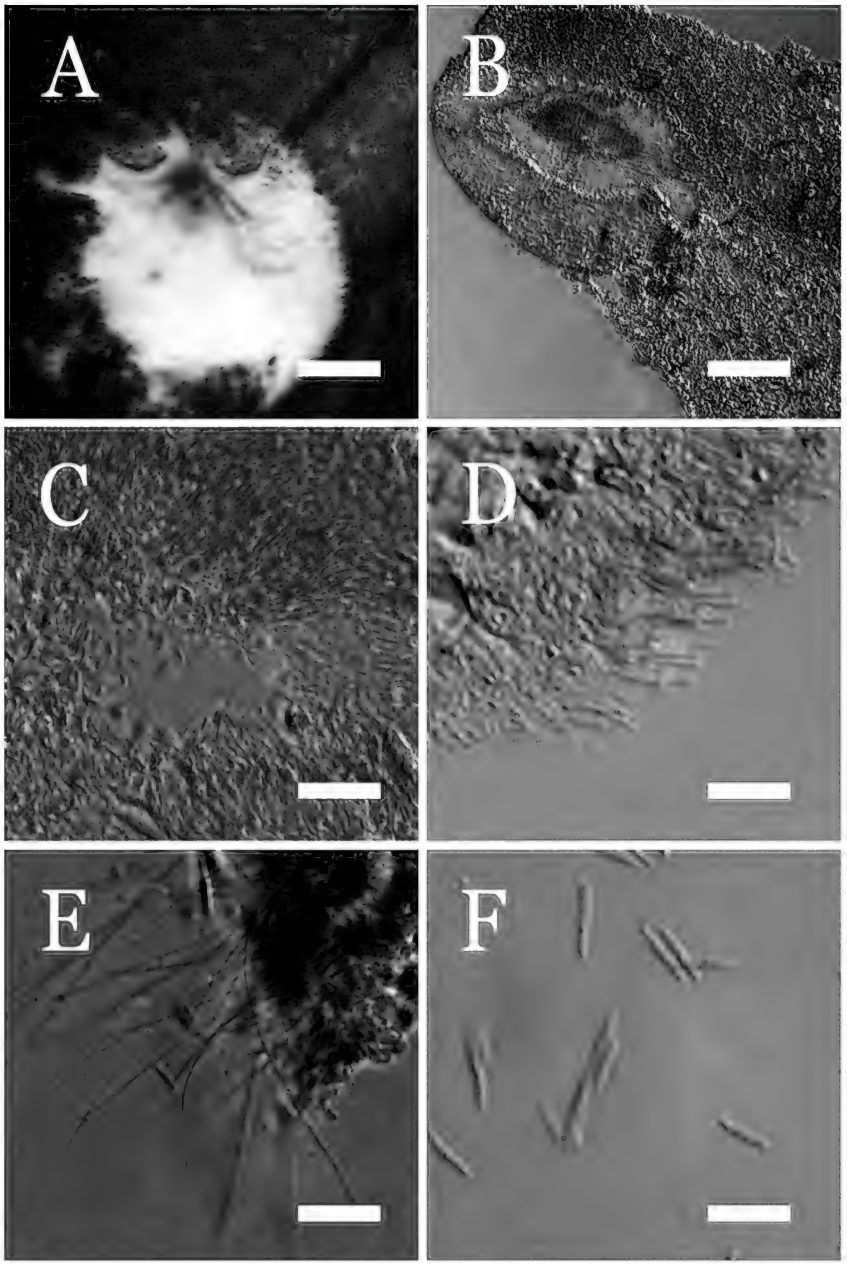

FIGURE 1. *Aschersonia conica*. A: Stroma; B: Pycnidium; C: Horizontal-section of a flask-shaped pycnidium; D: Conidiophores and conidiogenous cells; E: Paraphyses; F: Conidia. Scale bars: A = 1 mm; B–C = 100 μm; D–E = 30 μm; F = 10 μm.

presence of paraphyses and hypothallus. Two previously described species, *A. turbinata* Berk. and *A. juruensis* Henn. (Petch 1921, Chaverri et al. 2008), also have similarly shaped stromata. However, *A. turbinata* differs in having ovoid conidia that are longer and wider (10.5–11.2 × 4.5–5.0 µm), producing copious slime, and lacking paraphyses. *Aschersonia juruensis* differs in pulvinate stromata with sloping sides (convex), more conidiomata per stroma (>20), longer wider conidia (14.5–15.5 × 3.7–4.0 µm) that are ventricose with acute ends, and the absence of paraphyses.

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Clarkeinda trachodes (*Agaricales, Basidiomycetes*), first record from Bangladesh

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Abstract — An interesting agaric, *Clarkeinda trachodes*, is reported for the first time from Bangladesh. A full description, discussion, and illustrations are provided. The enigmatic agaric genus, currently known only from south and southeast Asia, is characterized by the presence of a fawn colored pellicle on the central pileus surface, a stipe with a superior annulus and basal volva, and thick-walled pigmented spores with a slightly depressed truncated apex. Our examination of a voucher specimen from Italy indicates that a recent report of *Clarkeinda* in Europe was based on a misidentified *Agaricus* collection.

Key words — *Agaricaceae*, distribution, morphology, taxonomy

Introduction

Taxonomic reports on macrofungi of Bangladesh are rare in the literature. As a part of an ongoing effort to document the macrofungi of South Asia, we report the presence of *Clarkeinda trachodes* from Bangladesh for the first time. We provide a detailed morphological description, photographs, and line drawings of this enigmatic agaric.

Materials & methods

During November 2009 and June 2010 specimens were collected several times in two different locations in Bangladesh: Bhawal National Park, Gazipur (24°45'N 90°50'E; altitude 20 m), and Akhanagar, Thakurgaon, in northern Bangladesh, (26°03N' 88°47'E; altitude 67 m). Specimens were deposited in the SAU Herbarium of Agarics Flora (SHAF; Plant Pathology Department, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh) and in the Kunming Institute of Botany Herbarium, Academia Sinica (KUN, with HKAS numbers), Kunming, Yunnan, China.

Fresh specimens were described and photographed in the field. Small tissue pieces were dried in silica gel and the remaining basidiomes were dried using an oven. Color codes follow Kornerup & Wanscher (1978).

Microscopical observations were made in mounts of H_2O, glycerin, 5% aqueous KOH, Congo red solution, and Melzer's reagent, and at least 20 basidiospores were measured for each of the six basidiomes collected. Q = average length/width ratio derived from each basidiospore measured. Line drawings of microstructures were made from the rehydrated specimens.

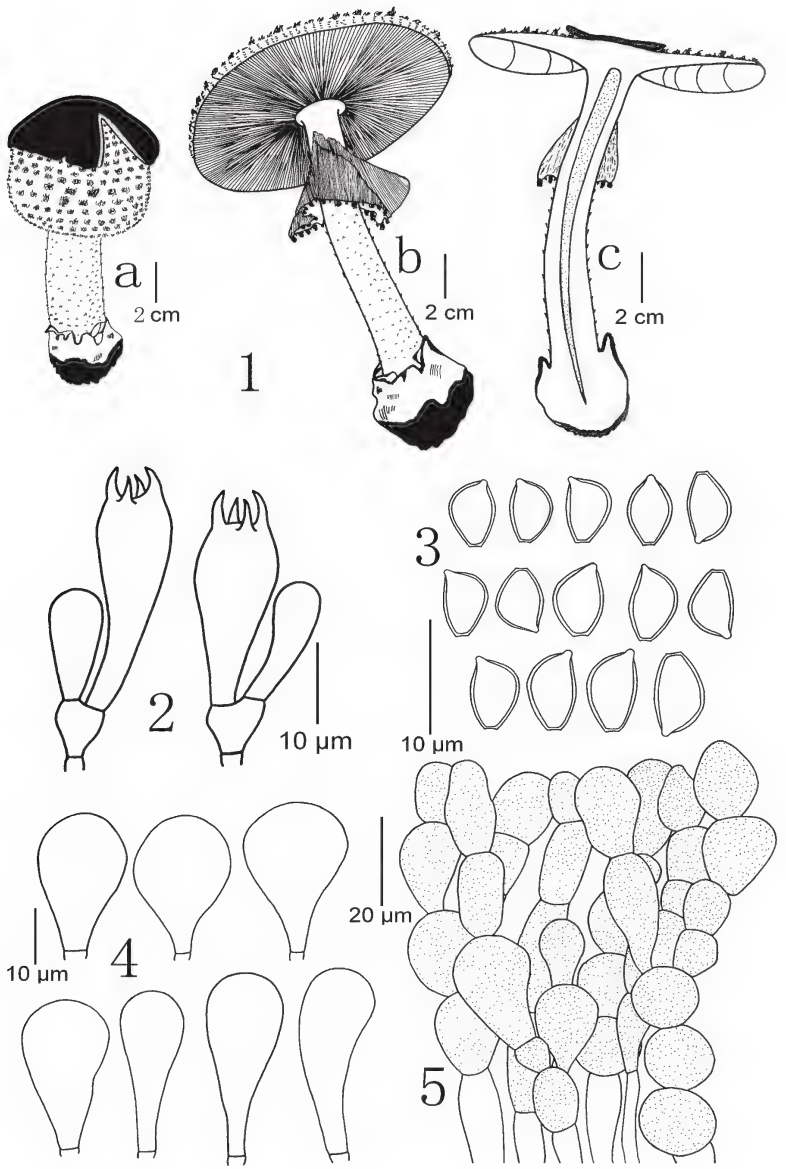
To prepare the basidiospores for scanning electron microscopy (SEM), silica dried basidiome fragments were mounted on aluminum stubs with double-sided tape, coated with gold palladium, and photographed with a Hitachi S–4800 SEM.

Taxonomy

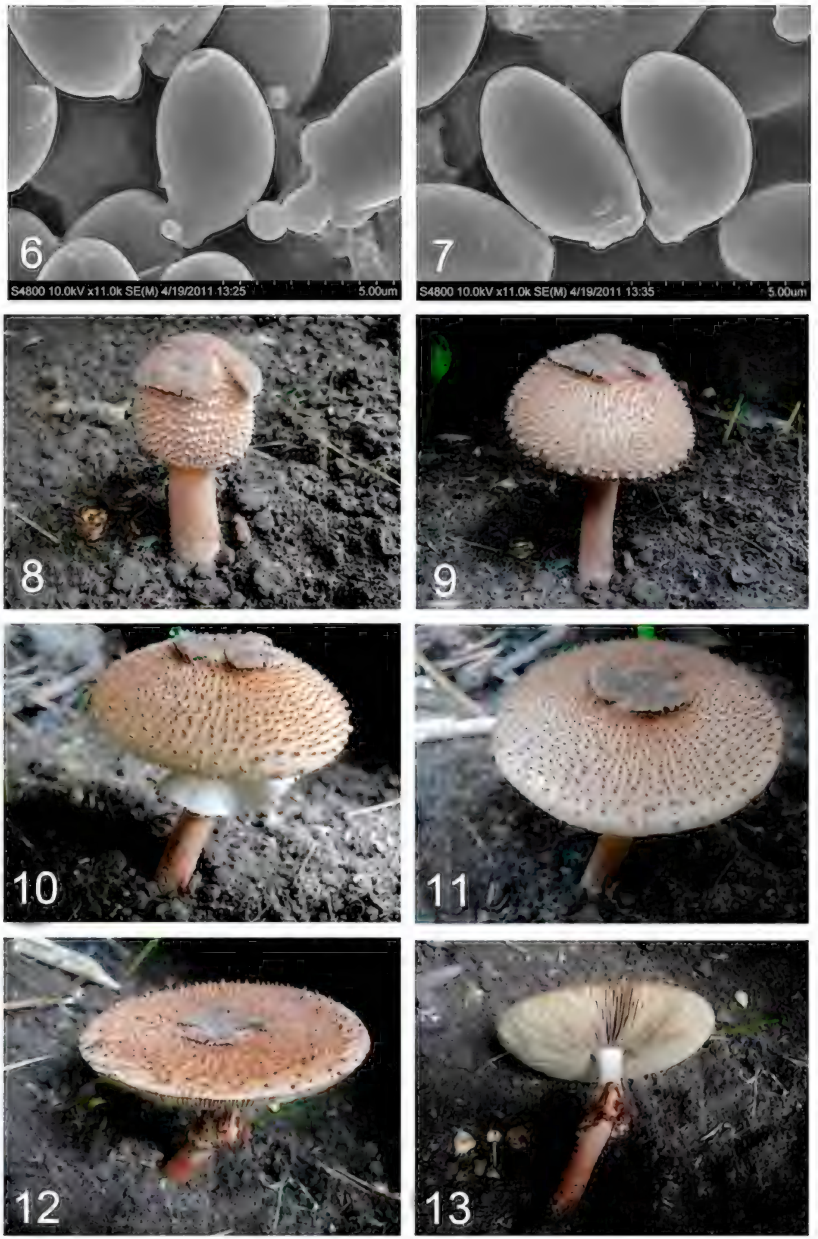
Clarkeinda trachodes (Berk.) Singer, Lilloa 22: 413, 1951. Figs 1-11

Basidiomata medium to large, fleshy. Pileus 80 to 110 mm in diameter, subglobose or hemispherical when young, and becoming convex to applanate at maturity; pellicle brown (6E8) to coffee (6E5-6) or chocolate brown (6E5, 6F5), thin to thick when young and somewhat brown to grayish brown (7E3-5) at maturity; the remaining surface covered with grayish brown (7E3-5) to vinaceous brown (6D8, 8E8) squamules, together with numerous, small, revolute and loosely floccose, brown squamules; context up to 9 mm thick in the center of the pileus, white, instantly turning reddish with exposure. Lamellae 47 × 10 mm, free and remote from stipe, white to dirty white when young, olive brown when mature, becoming red brown after bruising, crowded with lamellulae, margin entire, concolorous. Stipe 80–121 × (28–)31–47(–61) mm, central, subcylindrical, solid but fistulose in aged specimens; surface dirty white to white at the apex, light brown to brown toward the base, glabrous above the annulus, lower half densely covered with minute, brown, furfuraceous squamules. Annulus present on the upper part of the stipe but not the top, up to 18 mm, thick and membranous and remaining up to the maturity, sometimes detached from the stipe when dry, adaxial part glabrous with fine longitudinal striation but abaxial part rough with squamules. Volva present, grayish, dirty white to white, membranous, usually closely appressed to stipe and eventually inconspicuous. Spore deposit not obtained.

Basidia 18–26 × 6–9 μm, mostly clavate to subclavate, thin-walled, tetrasporic (occasionally 2- or 3-spored), bearing four short sterigmata, 1.1–2.3 × 0.8–1.2 μm, hyaline, smooth, lacking incrustations, clamp connection absent. Basidioles narrowly clavate to clavate. Hymenophoral trama interwoven, hyphae cylindrical to slightly inflated, up to 14 μm wide, thin-walled, hyaline, and without clamp connections. Basidiospores (Figs 3, 6–7) (5.0–)5.52–5.96(–6.8) × (3.4–)3.81–3.98(–4.3) μm, mean Q = (1.38–)1.42–1.54(–1.7), ovoid, sometimes broadly ellipsoid to ellipsoid, glabrous, thick-walled (up to 0.6 μm), apiculus



Figs 1–5: *Clarkeinda trachodes.* 1. Basidiome: (a) immature, (b) mature, (c) longitudinal section of mature fruiting body. 2. Basidia with basidioles. 3. Basidiospores. 4. Cheilocystidia. 5. Pileipellis.



Figs 6–13: *Clarkeinda trachodes*. 6–7. Basidiospores showing distinctly truncated and depressed apical germ pore under SEM. 8–13. Developmental stages of a basidiome fruiting in its natural habitat. (Photo: M.I. Hosen).

eccentric, apex or germinating pore prominent and truncate with slightly depressed, olive brown to dark, umber brown in deposit and maintaining the same color after desiccation for 1.5 years at room temperature, dextrinoid in Melzer's solution, not metachromatic in Cresyl blue. CHEILOCYSTIDIA 24–32 × 10–16 µm, abundant, scattered to more or less crowded, narrowly clavate, clavate to broadly clavate, obpyriform, hyaline, thin-walled, smooth, lacking incrustations, sometimes long pedicel and narrow up to 3.4 µm. PLEUROCYSTIDIA absent. PILEIPELLIS consisting of short branching chains of 4–7 cells, slightly interwoven, terminal cells 12–22.5 × 8–14 µm, dull brown vacuolar pigment inside the cells in glycerin, water and 5% KOH solutions, thin-walled, clavate, cylindrical, obpyriform to fusiform or spindle-shaped in rare cases, occasionally branching with lateral cells that are mostly clavate, basal cells nearly subglobose to clavate or cylindrical.

HABIT, HABITAT, DISTRIBUTION— The fleshy basidiomes of *C. trachodes* fruit as isolated individuals or in groups of two in disturbed habitats and at forest edges. Our collections were found along the roadside near a *Shorea robusta* (*Dipterocarpaceae*) forest in Bhawal National Park, Gazipur, and on bare soil near a bamboo fence around a village residence in Akhanagar, Thakurgaon, Bangladesh. Known also from China, India, Indonesia, Malaysia, and Sri Lanka.

SPECIMENS EXAMINED — **BANGLADESH. DHAKA DIVISION**: Gazipur, Bhawal National Park, 20 m a.s.l., 30 September 2009, M. I. Hosen 617, 619 (HKAS 62856, 62857); **RANGPUR DIVISION**: Thakurgaon, Akhanagar, 67 m a.s.l., 16 June 2010, M. I. Hosen 85–88 (HKAS 62852–62855).

ADDITIONAL MATERIAL EXAMINED — ***Agaricus* sp.**: **ITALY**, South Italy, on sandy ground next to *Castanea sativa*, 1100 m a.s.l., 13 October 1994, Carmine Lavorato (HKAS 49489, as *Clarkeinda trachodes*).

COMMENTS — *Clarkeinda trachodes* is distinguished by a large basidiome size, prominent chocolate or coffee brown to deep brown pellicle on the pileus disc surface, presence of an annulus, olive brown to umber brown spore deposit, slightly thick-walled spores with a truncated apex, and a context that changes from white to reddish brown when cut. Since Berkeley (1847) first described the species from Sri Lanka, it has been reported from south and southeast Asia by Petch & Bisby (1950, as *Chitoniella*), Leelavathy et al. (1981), and Pegler (1985, 1986). Yang (1991) has also reported it from the tropical region of Yunnan, China.

Examination of the specimen from Italy reported as *C. trachodes* by Lavorato & Contu (2002) shows the European record is based on a misidentified *Agaricus* specimen. The Italian specimen bears larger, ellipsoid basidiospores (7–8.5 × 4.5–5.5 µm) that lack a germ pore and truncated apex, and pileal squamules that represent a cutis of filamentous cylindrical hyphae 4–8 (–15) µm in diameter.

Vellinga et al. (2011) and our own sequence analyses (Ge unpub.) nest *Clarkeinda* phylogenetically in the *Agaricus* s.l. clade close to *Agaricus* and *Heinemannomyces* (genera within tribe *Agariceae*).

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Glomus cubense sp. nov., an arbuscular mycorrhizal fungus from Cuba

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Abstract —*Glomus cubense* (*Glomeraceae, Glomeromycetes*) was isolated from a lagoon vegetation area on a clay soil deposition environment in the vicinity of San José de las Lajas, Cuba. The species description is based on spore morphological parameters from in vivo pot cultures and molecular analyses. The new species is characterized by its small, generally irregular in shape, 20-48 × (24–)54-72 µm hyaline to faintly yellow spores that have a 2-layered spore wall and arise in clusters. Phylogenetic analyses of the rDNA ITS region and H+ATPase place the species into the *Glomeraceae* without close relatives among named *Glomus* species. *Glomus cubense* forms mycorrhizal associations with sorghum and leek plants under greenhouse pot-culture growing conditions and with a diversity of crop plants under field conditions. The name *cubense* refers to Cuba, the country where the species was found.

Keywords — *Glomeromycota*, molecular phylogeny

Introduction

Since 1973, numerous investigations on the diversity of arbuscular mycorrhizal fungi (AMF) have been performed in Cuba, and several species have been described and surveyed from this country (Herrera & Ferrer 1980; Ferrer & Herrera 1981, Herrera-Peraza et al. 2003). With respect to research and development on the selection of AMF strains with potential for use as bio-inoculants for agriculture and horticulture productions, AMF species populations were surveyed and propagated in pure cultures, characterized morphologically, and simultaneously tested for performance at stimulating

plant yields under both controlled and field conditions (Rivera et al. 2003, 2007; Herrera-Peraza et al. 2011). Among the AMF strains surveyed and tested, there was an undescribed species, here named *Glomus cubense*, which formed small, hyaline, 2-layered glomoid spores in clusters around roots. Morphological and molecular comparisons with other light-coloured glomoïd spore species (Błaszkowski et al. 2010) clearly distinguished *G. cubense* from previously described species. Once propagated in pot cultures, it showed high root colonization potential and growth benefits over a variety of crop plants tested. In the present paper, the fungus is proposed as a new arbuscular mycorrhizal species, *G. cubense,* in the *Glomeromycota.*

Materials & methods

AMF strain propagation and evaluation were conducted in the central greenhouse of the National Institute of Agricultural Sciences (INCA) in San José de las Lajas, Cuba. Species morphological and molecular characterizations were done at the Eastern Cereal and Oilseed Research Centre of Agriculture and Agri-Food Canada, Ottawa, Canada.

AMF sampling and propagation

Soil samples were collected at about 50 cm depth from a lagoon area at "San Rafael" Pond in "Las Papas" area (San José de las Lajas, Cuba), with a Haplic Cambisol (chromic) soil (IUSS Working Group WRB, 2006), a natural soil deposition environment occasionally flooded and mainly covered with *Cynodon nlemfuensis* and *Mimosa pigra.* Mixed soil samples were used to establish pot trap-cultures using *Sorghum bicolor* as host plant. After a four months growth period, spores of AMF were isolated by morphotypes and used as inoculant for the establishment of pure cultures in sterilized (121°C for 3 h during three days) clay substrate from a "Gley vértico cálcico" soil (Instituto de Suelos 1999) from "Guayabal" farm in San José de las Lajas, Cuba. Plants were fertilized and watered at regular intervals (Hewitt 1966). Pure strain cultures were subsequently maintained as reference culture on sorghum plants under semi-controlled greenhouse conditions.

AMF isolation and characterization

Spores were extracted from the substrate by wet-sieving (300–38 µm mesh sieves) and centrifugation in sucrose gradient (Dalpé & Hamel 2007). Spores were then manually isolated from the supernatant under the dissecting microscope (Olympus SZ-PT) by micropipetting. Plant roots were washed in water, bleached in 2.5% KOH and stained with fuchsin acid (0.01% in lacto-glycerol) (Kormanik & McGraw 1982). Once destained in lacto-glycerol, root sections were mounted on microscope slides in polyvinyl alcohol-lactic acid-glycerol (PVLG) (Omar et al. 1979), a mixture of PVLG and Melzer's reagent (1:1, v/v), or a mixture of PVLG and Cotton Blue (0.15% in lactic acid) (1:1, v/v). Spore morphology and spore wall architecture were based on examination of about a 100 spores. Color observations referred to the color chart code of the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM; http://invam.caf.wvu.edu/). Microscopic observations were performed with a Nikon Eclipse 800 compound microscope equipped with Nomarski differential

interference contrast optics and photographs were taken with a Nikon CoolPix 4500 digital camera. Nomenclature and AMF classification follow Schüßler & Walker (2010). Reference specimens were deposited at the Cuban National Herbarium, IES-CITMA (HAC) Cuba, and at the National Mycological Herbarium (DAOM) Canada.

Molecular analyses

Spore clusters were collected under a low magnification binocular, rinsed with ddH_2O and air-dried. DNA extraction was performed using the PrepMan Ultra (PMU) reagent (Applied Biosystems). A ratio of 10 spores/µl of PMU reagent were crushed using sterile plastic micropestle. Initial PCR reaction for H^+ ATPase was performed following Sokolski et al. (2010) with primer GmossATPup1058 (5′-CGG TAC TTT GTT CTG ATA AGA C-3′) and primer mix GintraATPlo2663, GmossATPlo2663, GcoroATPlo2663. Three nested PCR were performed using primers pairs:1) GintraATPup1104, GmossATPup1104 with GintraATPlo2663, GmossATPlo2663, GcoroATPlo2663 (Sokolski et al. (2010), 2) *Glomus*ATPup1150 (5′-WTC HGC YGA ACC TGG TGC-3′) with *Glomus*ATPlo2557 (5′-CTK GHT TTW GAW GGC CAK AAT-3′) at 52°C or 3) with GintraATPlo2663, GmossATPlo2663, GcoroATPlo2663 at 58°C. The ITS rDNA primers pair used were: GLO2A-forward (5′-CGT AAC AAG GTT TCC GTA GG-3′), GLO2R-reverse (5′-GCG GGT ACT CCT ACC TGA TT-3′)(Sokolski et al. 2010). PCR products were cloned using the TOPO TA Cloning® Kits (Invitrogen, USA). Cloned products were purified using the GenElute™ Plasmid Miniprep Kit (Sigma, USA). Cloning and DNA extraction were performed following the instructions of the manufacturer for rDNA ITS. Between two and eight rDNA ITS molecular clones (av. four) were selected. H+ ATPase and ITS were sequenced using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif.) and run on an ABI 3100-Avant automated sequencer (Applied Biosystems/ Hitachi). Sequence editing was done using SeqMan (DNASTAR, www.dnastar.com/), resulting in 1305 bp and 537, 546 and 549 bp alignments, respectively. The sequences obtained were compared with those in the GenBank databases using the BLAST program. Sequences retrieved from GenBank and those in this study were aligned by Muscle 3.8.31 and a neighbour-joining tree inferred using MEGA 5.0 (Tamura et al. 2011) with 1000 replicates. The best-fit model (General Time Reversible plus Gamma) was selected using the web-based MODELTEST 3.7 (Posada 2006). Sequences were deposited in GenBank under accession numbers JF510464 for H+ ATPase and JF692724, JF692725, JF692726 for ITS rDNA.

Taxonomy

Glomus cubense Y. Rodr. & Dalpé, **sp. nov.** PLATES 1–2

MYCOBANK MB561650

Sporocarpia ignota. Sporae singulares vel aggregatae. Sporae hyalinae vel luteolae, ovoideae, vel irregulares, 20–48 × (24–)54–82 µm, raro globosae, 24–54 µm diam. Sporae tunica stratis duobus; stratum exterius hyalinum, subflexuosum, ad 0.8–1.0 µm crassum, stratum interius hyalinum vel luteolum, 0.8–1.7 µm crassum, rigidum. Hyphae sustinentes hyalinae, rectae, subcylindricae vel subinfundibuliformes, 4–10 µm crassae ad basim sporae. Porus apertus, raro septo curvo clausus cum strato interiore. Mycorrhizas vesicular-arbusculares formantes.

Type: Cuba: "Las Papas" area, San José de las Lajas, from a Fersialítico Pardo rojizo mullido carbonatado soil in "San Rafael" Pond. (**Holotype**, permanent slides mounted in PVLG deposited at HAC Cuban National Herbarium, Instituto de Ecología y Sistemática, CITMA, La Habana, Cuba; **isotype**, DAOM 241198). Genbank JF510464, JF692724, JF 692725, JF692726.

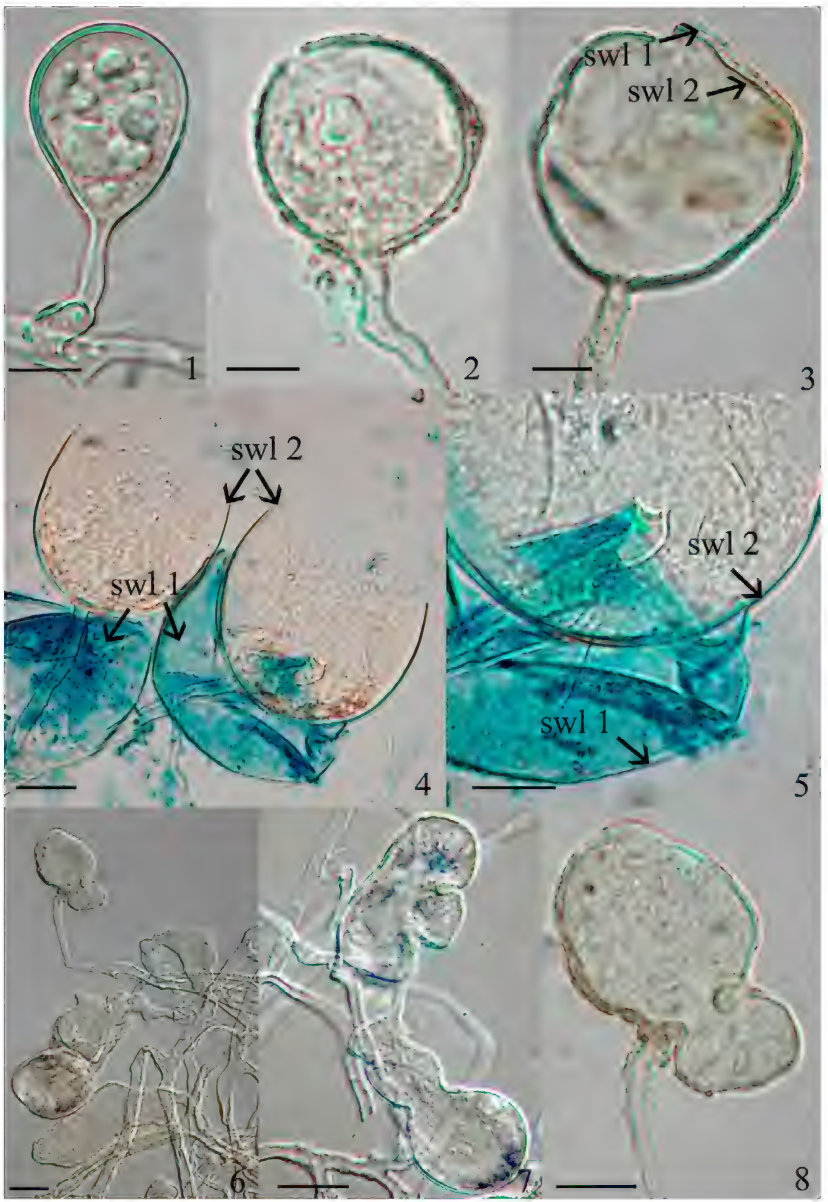
Etymology: Latin, *cubense*, in reference to Cuba, the country where the species was found.

Sporocarp unknown, spores mostly found in loose aggregates of 3 to 12 spores arising blastically at the top of sporogenous hyphae branched from a parent hypha continuous with an extraradical mycorrhizal hypha, rarely single in the soil. Spores (FIGS 1–8) hyaline to very light yellow (0/0/40/0); ovoid, ellipsoid, pyriform to irregular in shape 20–48 × (24–)54–82 µm, rarely globose, 24–54 µm in diameter with one subtending hypha. Spore wall composed of two layers. Spore wall layer 1 (Swl 1) hyaline, permanent, flexible to semi-flexible, 0.8–1.0 µm thick, with a smooth surface, easily separating from layer 2 in crushed spores. Swl 2 hyaline to very pale yellow, rigid, 0.8–1.7 µm thick. Spore wall layers do not react to Melzer's reagent, spore wall layer 1 stained light blue in Cotton Blue. Subtending hypha concolorous to spores; cylindrical to slightly flared, straight or recurved; 4–10 µm wide at spore base. Wall of subtending hyphae hyaline to very light yellow; 1.6–2.0 µm thick at the spore base; composed of two layers continuous with spore wall layers 1 and 2. Pore usually open, occasionally closed by a curved septum located 6–8 µm from the spore base or sometimes occluded by thickening of spore wall layer 2.

Intraradical structures — *G. cubense* forms vesicular-arbuscular mycorrhizae with intracellular ellipsoid hyphal coils (FIG. 11), globose, 23–38 µm, to ovoid to irregular vesicles, 18–25 × 20–60 µm (FIGS 10, 12) and a few arbuscules.

Mycorrhizal association — In the field, found associated with typical lagoon vegetation plants such as *Cynodon nlemfuensis* and *Mimosa pigra*. In field trials, developed symbiosis with a diversity of crop plants: avocado trees, banana, cassava, corn, cucumber, forages (*Brachiaria decumbens* and *Panicum maximum*), guava, malanga, mango, pepper, plantain, potato, rice, soybean, bean, sweet potato, tobacco, tomato, wheat, yam (Rivera et al. 2007). In the greenhouse, pot cultured for several years with sorghum (*Sorghum bicolor* and leek (*Allium porrum*) plants.

Phylogenetic position — The ITS rDNA and H+ ATPase analyses (Figs 13–14) placed *Glomus cubense* in the genus *Glomus* sensu Schüßler & Walker (2010) in the family *Glomeraceae* with no close relationship with known AMF species of this family. Moreover, no environmental sequences were found to be closely related to *G. cubense*.



Figs. 1–8. Spores of *Glomus cubense* (DIC) from pot-cultures. 1. Juvenile spore with hyphal attachment. 2. Mature spore with hyphal attachment. 3. Mature spore slightly crushed showing spore wall layers 1 and 2 (swl1, swl2). 4–5. Mature spores with spore wall layer 1 stained with Cotton Blue compared to spore wall layer 2. 6–8. Irregular shaped spores with subtending hyphae. Bars: 1–3 =10 μm; 4–8 = 20 μm.

Figs. 9–12. *Glomus cubense* fungal structures inside colonized roots (DIC). 9. Entry point along a root. 10. Vesicles. 11. Hyphal coil inside cortical root cell. 12. Vesicles inside cortical root cell. Bars: 9–12 = 20 µm.

Specimens examined: **CUBA.** Spores and roots isolated from *Sorghum bicolor* pot cultures, grown in greenhouse on sterilized clay substrate (Gleyic Calcic Vertisol soil (IUSS Working Group WRB, 2006) with the following chemical characteristics: Na^{+} 0.81, K^{+} 0.42, Ca^{2+} 48.1, Mg^{2+} 6.6 (cmol/kg^{-1}); P 30.8 (ppm); organic matter 0.84 (%); pH

8.3. **CANADA.** Spores and roots isolated from *Allium porrum* pot cultures, grown on cultivated Brown Chernozem soil (Saskatchewan, Canada) with a loamy sand texture and the following chemical characteristics after sterilization: NH_4-N 19.7 and NO_3-N 14.1 (mg. kg^{-1}), P 21.3 and K^+ 324.5 (mg. kg^{-1}), pH 6.5 and EC 0.48 mS.

OTHER SPECIMENS EXAMINED: Type specimens of *G. bistratum* Błaszk. et al., *G. perpusillum* Błaszk. & Kovács, provided by J. Błaszkowski, *G. canadense* (Thaxt.) Trappe & Gerd. (FH 5045), *G. fragile* (Berk. & Broome) Trappe & Gerd. (K Berkeley 1182) and of *G. viscosum* T.H. Nicolson (PI Holotype)

DISTRIBUTION AND HABITAT. *Glomus cubense* is only known from a single location: "Las Papas" area, San José de las Lajas, Cuba (22°57′41″N 82°09′04″W). Spores were originally isolated from Haplic Cambisol (chromic) soil collected at ca. 50 cm depth. The soil corresponds to a "Fersialítico Pardo rojizo mullido carbonatado" soil (Instituto de Suelos, 1999). The species was propagated in pot-culture using the same type of soil originating from the same area in Cuba.

Notes

The main distinctive morphological properties of *G. cubense* are its small hyaline to very light yellow, usually irregularly-shaped spores (FIGS 1–2, 6–8) and its 2-layered spore wall (FIGS 3–5) composed of a permanent, flexible to semi-flexible outer layer staining distinctly in Cotton Blue (FIGS 4–5) and a rigid inner layer, non-reactive to Melzer's reagent. Under the dissecting microscope, *G. cubense* may resemble most glomoid small-sized and pale-colored spores as treated in the Błaszkowski et al. (2010) identification key. However, when observed under the compound microscope, only six named species produce glomoid pale coloured, inamyloid and 2-layered spores that are smaller than 80 µm. These are *G. bistratum* (Błaszkowski et al. 2009b), *G. cerebriforme* McGee (McGee 1986), *G. indicum* Błaszk. et al. (Błaszkowski et al. 2010), *G. minutum* Błaszk. et al. (Błaszkowski et al. 2000), *G. pallidum* I.R. Hall (Hall 1977; Oehl et al. 2003), and *G. perpusillum* Błaszk. & Kovács (Błaszkowski et al. 2009a).

The *G. cerebriforme* and *G. perpusillum* spore wall layer 1 is laminate (vs. flexible in *G. cubense*) and much thicker than spore wall layer 2 (vs. opposite in *G. cubense*). Additionally, spore wall layer 2 of *G. perpusillum* stains in Melzer's reagent (vs. no staining in *G. cubense*). In the spore wall of the other species listed above, layer 1 is a permanent structure, semi-flexible to rigid, thinner than layer 2 as in *G. cubense* but layer 2 is laminate. The *G. minutum* spores are only hyaline and of globose to subglobose shape (vs. hyaline to very pale yellow, and mostly of irregular shape in *G. cubense*). While spore wall layer 1 easily separates from spore wall layer 2 in freshly crushed *G. cubense* spores, that of *G. minutum* generally remains adherent to the laminate spore wall 2. In *G. minutum*, spore wall layer 1 sometimes swells and separates from spore wall layer 2, but only when spores are mounted in lactic acid-based mountants for at least some hours.

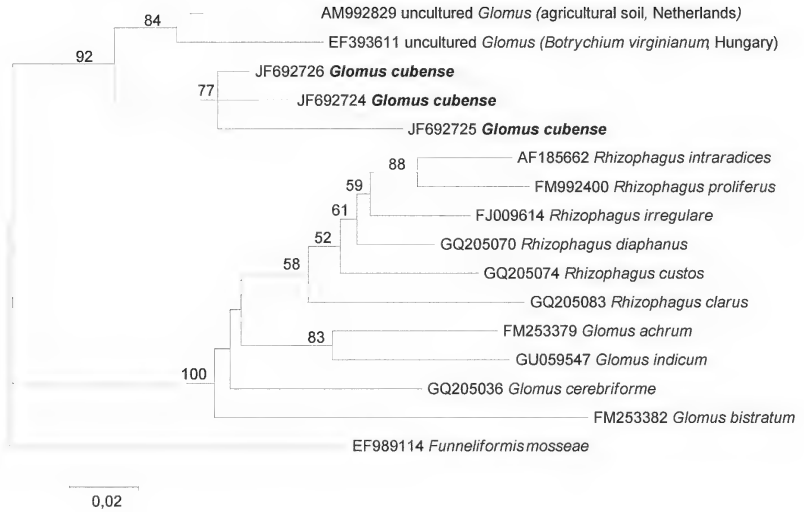


FIG.13. BIONJ tree based on an alignment of the ITS sequences obtained from *Glomus cubense* and related representative sequences from GenBank. Branch support values (only values exceeding 50% are given) on branches refer to BIONJ bootstrap (using GTR + G distances) and maximum likelihood. Scale indicates nucleotide substitution per site.

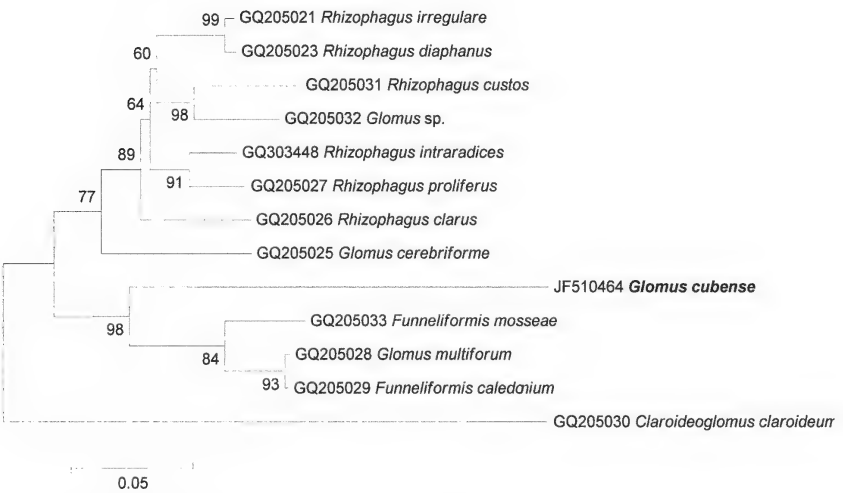


FIG.14. BIONJ (Neighbour-joining) tree based on an alignment of H+ ATPase sequences of *Glomus cubense* and of related representative sequences from GenBank. Branch support values (only values exceeding 60% are given) on branches refer to BIONJ bootstrap (using GTR + G distances) and maximum likelihood. Scale indicates nucleotide substitution per site.

Glomus microaggregatum Koske et al. also produces small, 2-layered spores frequently irregular in shape, but the spores are much darker-colored (yellow to brownish yellow), their spore wall is thicker (up to 4 µm vs. up to 2.7 in *G. cubense*) and comprises a rigid outer layer and a flexible inner layer (vs. flexible to semi-flexible outer layer and rigid inner layer in *G. cubense*).

The spore wall of *G. fragile* and *G. canadense* also consists of a hyaline outer layer and a pale yellow inner layer. However, observation of their respective type specimens (K Berkeley 1182, FH 5045) revealed that the inner spore wall layer in both species is thicker than that of *G. cubense* and laminate (Gerdemann & Trappe (1974).

Young or small-sized clustered spores of *Glomus viscosum* may resemble those of *G. cubense,* but they usually are globose, have a 3-layered spore wall, of which layer 3 is distinctively laminate.

Compared with *G. cubense,* glomoid spores of *Paraglomus* spp are hyaline to pale cream, they form only singly (vs. in cluster in *G. cubense*) and their spore wall layers clearly differ phenotypically from those of *G. cubense* (Błaszkowski et al. 2010).

Ambispora spp. differentiate hyaline but much larger spores (Walker et al. 2007).

The phylogenetic analyses of sequences of the H+ATPase gene clearly support morphological analyses and separate *G. cubense* from the few sequences actually available and confirmed the efficiency of earlier published primers for sequencing *Glomus* species (Sokolski et al. 2010). The analyses of ITS region confirm that the species *G. cuben*se is a member of the genus *Glomus* sensu Schüβler & Walker (2010) in the family *Glomeraceae* without having any close described relatives. When comparing ITS sequence data, the best Blast alignment with Genbank database sequences indicated a maximum of 83 and 85% similarity with an as yet uncultured *Glomus* sp. thus supporting the status of *G. cubense* as a distinct species.

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Two new species of *Exserticlava* and *Spiropes* on decaying wood from Guangdong, China

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Abstract — Two new anamorphic fungi, *Exserticlava manglietiae* on decaying branches of *Manglietia chingii* and *Spiropes terminaliae* on decaying branches of *Terminalia mantaly,* are described and illustrated. The specimens were collected from subtropical rainforests in Guangdong Province, China. Morphological differences between these two fungi and similar species are summarized.

Key words — anamorph, hyphomycete, microfungi, systematics

Hughes (1978) introduced *Exserticlava* to accommodate *Cordana vasiformis* Matsush. and *C. triseptata* Matsush., two species originally described on wood and bamboo from Japan (Matsushima 1975). *Exserticlava* is characterized by thick-walled, distoseptate conidia from polyblastic, funnel-shaped conidiogenous cells borne on mononematous, simple conidiophores (Tsui et al. 2001a). *Exserticlava* is similar to *Cacumisporium* Preuss and *Chloridium* Link in having sympodulo-phialides (Cole & Samson 1979) and holoblastic conidia formed from the apex of polyblastic conidiogenous cells. *Cacumisporium* differs from *Exserticlava* in euseptate conidia and cylindrical conidiogenous cells (Tsui et al. 2001b). Conidia in *Chloridium* are hyaline and aseptate (Gams & Holubová-Jechová 1976).

Ciferri (1955) established *Spiropes* with *S. guareicola* (F. Stevens) Cif. as type species. The genus is characterized by macronematous unbranched sometimes geniculate conidiophores, polyblastic, terminal and intercalary, sympodial cicatrized conidiogenous cells, numerous and conspicuous scars, and solitary acropleurogenous most commonly obclavate conidia with 1–9 transverse septa or distosepta.

During continuing research on saprobic fungi in subtropical forests of Guangdong Province, China, we collected two hyphomycetes that possess the morphological characteristics of *Exserticlava* and *Spiropes*. They differ morphologically from any described species and are, therefore, proposed as new to science. The type specimens are deposited in HSAUP (Herbarium of the Department of Plant Pathology, Shandong Agricultural University) and HMAS (Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences).

Exserticlava manglietiae S.C. Ren & X.G. Zhang, **sp. nov.** FIG. 1

MycoBank MB563099

Conidiophora macronematosa, nonramosa, erecta, laevia, atro-brunnea, apicem versus pallidiora, 6–11-septata, 360–490 × 5–8.5 µm. Cellulae conidiogenae polyblasticae, integratae, terminales, proliferationae sympodialiter. Conidia acrogena, holoblastica, solitaria, laevia, ellipsoidea, 1-distoseptata, pallide brunnea, 14–16 × 9–11 µm, ad basim truncata.

Type: CHINA. Gungdong Province: subtropical forest of Nanling, on dead branches of *Manglietia chingii* Dandy (*Magnoliaceae*), 10 Dec. 2010, Sh.C. Ren (**Holotype**, HSAUP H8363; **isotype** HMAS 146154).

Etymology: in reference to the host genus, *Manglietia*.

Colonies effuse, brown. Mycelium partly immersed, partly superficial, composed of septate, smooth, brown, branched hyphae. Conidiophores macronematous, mononematous, unbranched, erect, straight or slightly flexuous, smooth, thick-walled, dark brown, paler towards the apex, 6–11-septate, 360–490 × 5–8.5 µm, terminally swollen, 7.5–11 µm wide. Conidiogenous cells polyblastic, integrated, terminal, clavate, proliferating sympodially and producing a cluster of conidia. Conidial secession schizolytic. Conidia acrogenous, holoblastic, solitary, dry, smooth, ellipsoid, thick-walled, 1-distoseptate, very pale brown, 14–16 × 9–11 µm, base truncate.

Notes: *Exserticlava* species are separated primarily based on conidial shape and number of septa (Holubová-Jechová 1990). A key to species and a synopsis of their characters were provided by Tsui et al. (2001a). Of the six previously described *Exserticlava* species, *E. manglietiae* resembles *E. globosa* V. Rao & de Hoog (Rao & de Hoog 1986) in possessing concolorous, thick-walled conidia with a strictly median septum, but *E. globosa* produces spherical larger (17 × 21 µm diam) verrucose conidia. *Exserticlava uniseptata* Bhat & B. Sutton and *E. yunnanensis* L. Cai & K.D. Hyde also produce 1-septate conidia, but *E. uniseptata* can be distinguished by its broadly obovoid, larger conidia (15–20 × 11–14 µm) with a smaller basal cell and *E. yunnanensis* differs in hemispherical, pale brown to subhyaline, larger conidia (16–22 × 10–13 µm) with a smaller apical cell.

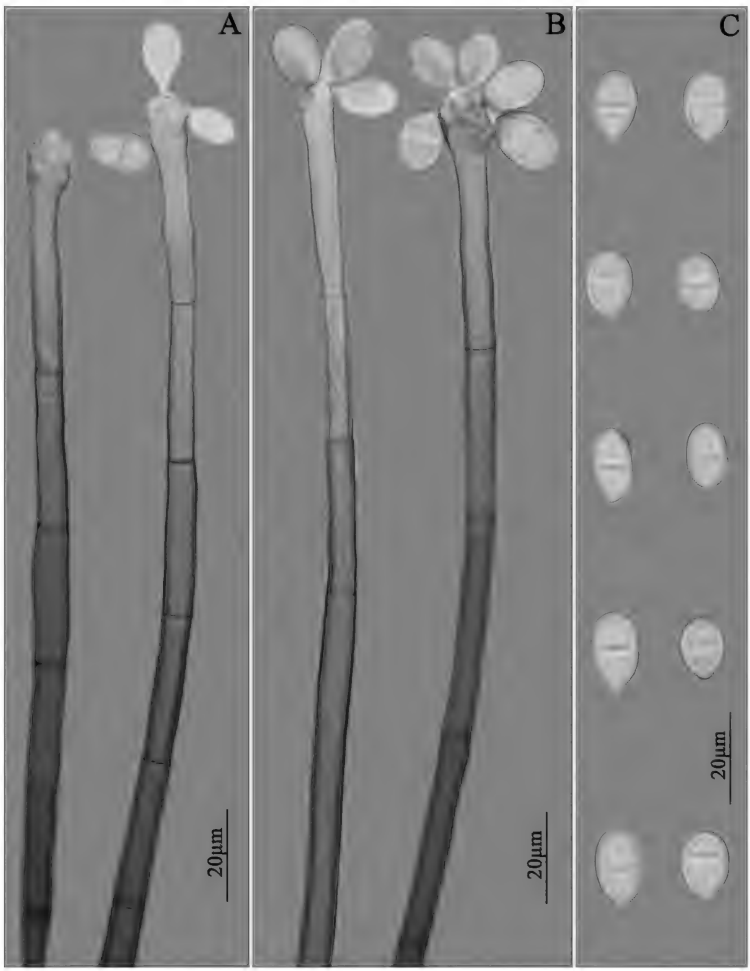

Fig. 1. *Exserticlava manglietiae*.
A–B. Conidiophores, conidiogenous cells and conidia. C. Conidia.

Spiropes terminaliae S.C. Ren & X.G. Zhang, **sp. nov.** Fig. 2

MycoBank MB563100

Conidiophora singula vel fasciculate, superus fertilis parte geniculatis efformata, usque 340 μm longa, basi 4.5–6.5 μm crassa, apice 7–9 μm crassa. Cellulae conidiogenae polyblasticae, integratae, terminales et intercalares, proliferationes sympodialite. Conidia acropleurogena, solitaria, late fusiformia vel oblonga, 5–7-distoseptata, laevia, 27–32 × 8.5–10.5 μm.

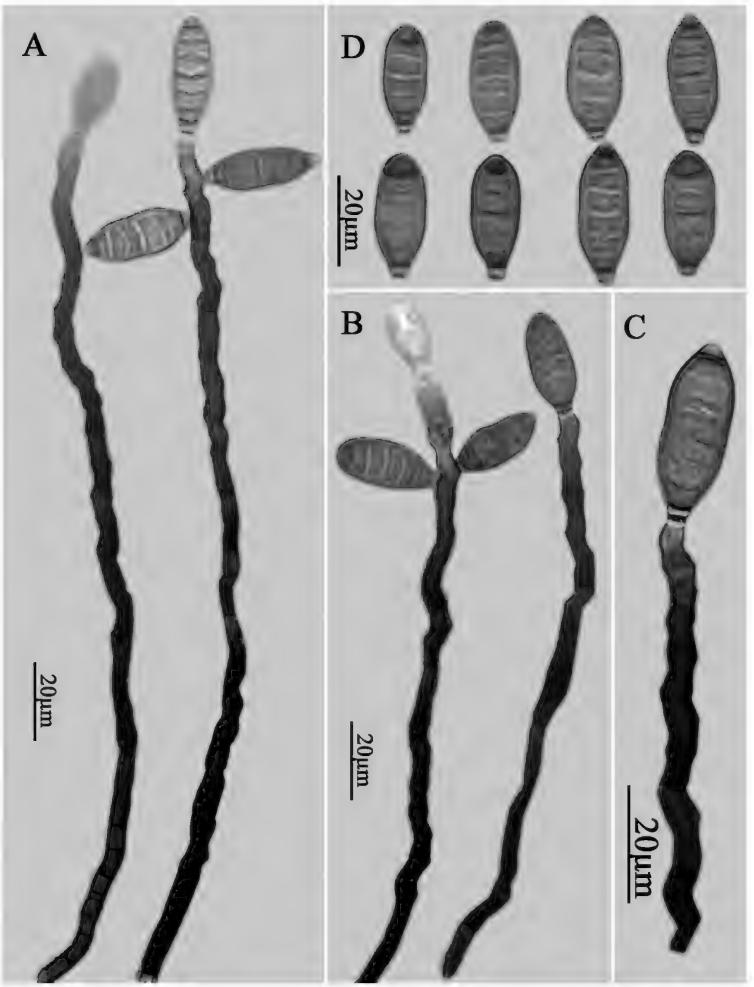

Fig. 2. *Spiropes terminaliae*.
A–C. Conidiophores, conidiogenous cells and conidia. D. Conidia.

Type: CHINA. Gungdong Province: subtropical forest of Nanling, on dead branches of *Terminalia mantaly* H. Perrier (*Combretaceae*), 15 Dec. 2010, Sh.C. Ren (**Holotype**, HSAUP H8218; **isotype**, HMAS 146155).

Etymology: in reference to the host genus, *Terminalia*.

Colonies on natural substratum effuse, dark blackish brown to black, hairy. Mycelium partly immersed and partly superficial, composed of branched, pale brown, smooth, septate, 2–4 μm thick hyphae. Conidiophores arising singly

or in groups terminally and laterally on the hyphae, erect, sterile lower part straight or flexuous, upper fertile part with zigzag proliferation, sometimes with geniculate fertile regions separated by sterile areas, brown to dark brown, paler toward the apex, with numerous dark conidial scars, 6–14-septate, up to 340 µm long, 4.5–6.5 µm wide at the base, 7–9 µm wide at the apex. CONIDIOGENOUS CELLS polyblastic, integrated, terminal and intercalary, sympodial, cylindrical, cicatrized, scars conspicuous. CONIDIA holoblastic, acropleurogenous, solitary, dry, broadly fusiform to oblong, brown to dark brown, smooth, 5–7-distoseptate, 27–32 µm long, 8.5–10.5 µm wide, 2.5–3.5 µm wide at the truncate base.

NOTES: Of the known *Spiropes* species, *S. terminaliae* is closely related to *S. guareicola* in possessing macronematous unbranched conidiophores where the upper fertile part has geniculate proliferations and distoseptate conidia. However, *S. terminaliae* differs from *S. guareicola* in conidial shape, size, and the number of septa. The conidia of *S. guareicola* are broadly fusiform, much larger (25–52 × 10–13 µm), and with fewer septa (3–5).

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Discovery of *Geastrum xerophilum* from the Neotropics

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Abstract — *Geastrum xerophilum,* a xerophytic species found in the Brazilian semi-arid region, is reported for the first time from the Neotropics. Descriptions, taxonomic remarks, and SEM-photos are provided.

Key words — Brazil, gasteroid fungi, *Geastraceae,* taxonomy

Introduction

The biological diversity of the Neotropical region is still poorly known, when compared with the Palearctic and Nearctic regions. Nevertheless, it is considered a reference area in terms of rich species diversity (Olson et al. 2001, Sodhi & Ehrlich 2010), encompassing megadiverse countries such as Brazil (Brooks 2006).

Most *Geastrum* species commonly grow in shady, humid habitats (Sunhede 1989), but some taxa, such as *G. xerophilum,* adapt to desert environments (Bates 2004). The diversity of these fungi in semiarid Brazilian biomes has always been underestimated and is practically unknown (Drechsler-Santos et al. 2008). Only since the turn of the century have research agencies in Brazil begun to sponsor the study of fungal diversity in the semiarid region of the country. The implementation of the Semiarid Biological Research Program (PPBio-Semi-Árido) has led to an increase in the study of these environments.

Material & methods

The studied material was collected in the city of Caicó, Rio Grande do Norte, Brazil. Macroscopic observations were made from fresh and dried materials. Color references

follow Kornerup & Wanscher (1978). For light microscopy, free-hand sections were mounted in 5% (w/v) KOH. Basidiospore surface features were observed by SEM from a small portion of the gleba, which was dusted onto double-sided adhesive tape on a specimen holder, coated with platinum (Monthoux 1982), and examined with a Philips - XL30 microscope. Twenty randomly selected basidiospores were measured using an ocular micrometer and scanning electron microscopy (SEM), and all spore measurements included surface ornamentation. Statistic measures of spore length and width are given as $x \pm$ SD (arithmetic mean ± standard deviation) and "Qm" (the ratio of mean spore length to width). The specimens are deposited in the Herbarium of the Federal University of Rio Grande do Norte (UFRN), Brazil.

Results

Geastrum xerophilum Long ex Desjardin, Pacific Science 65: 493 (2011).

Figs 1–3

Expanded basidiome saccate, 20 mm wide × 13 mm tall. Exoperidium splitting into 7 rays, non-hygroscopic. Rays involute rolling up under the endoperidium. Mycelial layer brown (6E4), felted, thin, encrusted with sand, persistent. Fibrous layer grayish brown (6D3), papery, thin. Pseudoparenchymatous layer dark brown (6F4), thick, rigid, persistent. Endoperidial body 16 mm wide × 8 mm tall (including peristome), stalked, depressed globose, grayish orange (5B3) to brownish orange (5C3), furfuraceous becoming glabrous with age. Apophysis discrete. Stalk <2 mm tall, concolorous with the endoperidium. Peristome plicate becoming lacerated with age, not delimited, concolorous with endoperidium, applanate. Gleba brown (6F5).

Basidiospores globose to subglobose 4.3–6.3 µm × 4.3–6.3 µm [$x = 5.6 \pm 0.7 \times 5.6 \pm 0.7$, Qm = 1.0, n = 20], with pedicel rudimentary, densely verrucose, yellowish brown in 5% KOH. Eucapillitium 3.8–6.3 µm diam., glabrous to lightly encrusted, lumen absent, walls >1 µm thick. Mycelial layer composed of thin sinuous-walled hyphae, lumen absent, 2.5–5.0 µm diam., yellow to hyaline in 5% KOH. Fibrous layer composed of thin straight walled hyphae, lumen absent, 1.6–3.8 µm diam., yellow to hyaline in 5% KOH. Pseudoparenchymatous layer composed of sublobose, citriform to elongated hyphae, 16.5–31.7 µm diam. × 12.7–33.0 µm in length, walls >1 µm thick, hyaline to pale yellowish in 5% KOH.

Substrate —Solitary in sandy soil.

Specimen examined: **BRAZIL. Rio Grande do Norte**: Caicó, 06°14′302″S, 37°02′958″W, 163 m a.s.l., 29.V.2010, col. B.D.B. Silva (UFRN 1508).

Taxonomic remarks — *Geastrum xerophilum*, a species typical of xerophytic environments, was collected in Brazil's Caatinga domain. The collection site is characterized by xerophilous vegetation and semiarid climate with low irregular rainfall and high temperature and evapotranspiration (Leal et al. 2005). The

FIGURE 1. Basidiomes of *Geastrum xerophilum*.

species is morphologically similar to *G. campestre* Morgan, which differs in its hygroscopic rays, delimited peristome, and larger spores (6.5–8.0 µm diam.) (Sunhede 1989). *Geastrum kotlabae* V.J. Staněk is another proximate species, but it exhibits strongly hygroscopic rays bent around the endoperidium and a detached mycelial layer and sessile endoperidium (Sunhede 1989, Bates 2004). Previously the distribution of *G. xerophilum* was thought to be restricted to North America, with records from Arizona (Bates 2004), New Mexico (Ponce de Leon 1968) and Mexico (Esqueda et al. 1995, 2009, Moreno et al. 2010), and to Hawaii (Smith & Ponce de Leon 1982, Gilbertson et al. 2001, Hemmes & Desjardin 2011). Therefore *G. xerophilum* is recorded here for the first time for the Neotropical region.

Recently, *G. xerophilum* was recognized as an invalid name and validated by Hemmes & Desjardin (2011) because Long's (1942) original description lacked a Latin diagnosis (McNeill et al. 2006: Art. 36.1).

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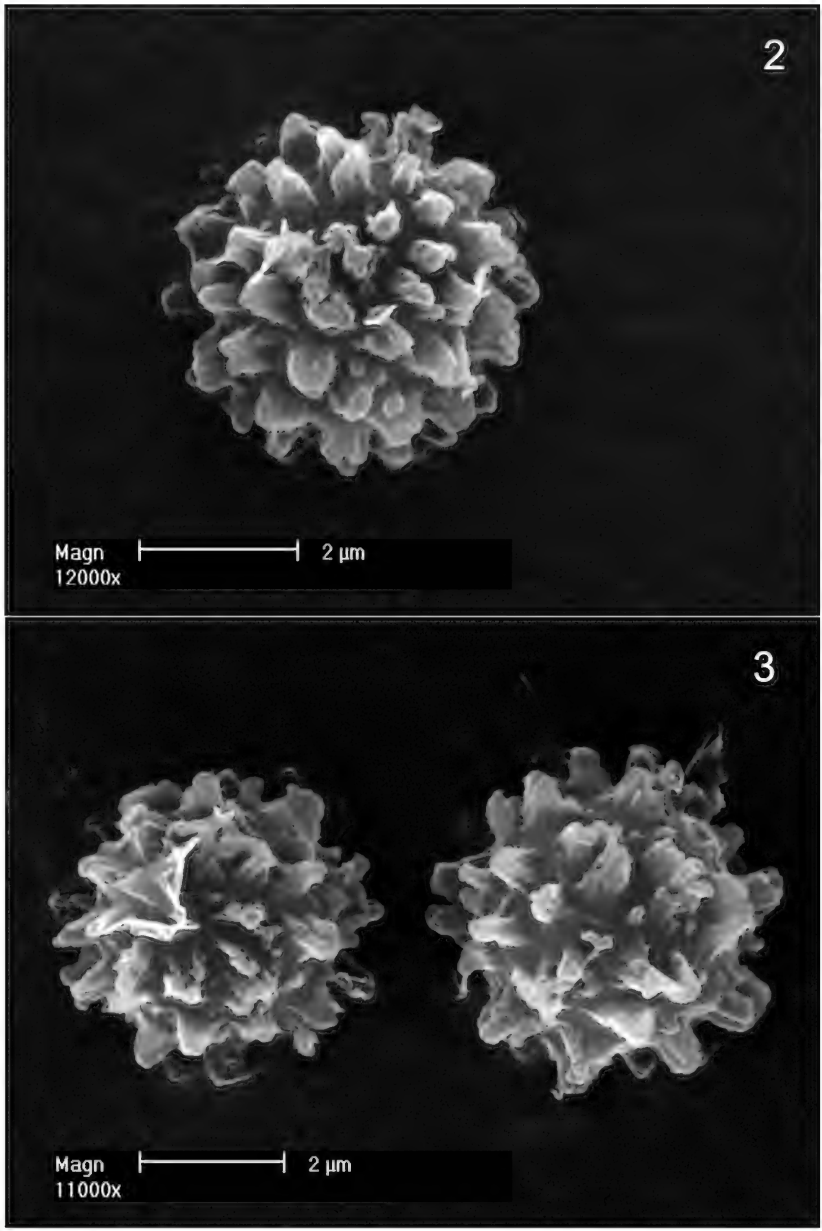

FIGURES 2–3. *Geastrum xerophilum*: basidiospores under SEM.

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A preliminary ITS phylogeny of *Melanoleuca* (*Agaricales*), with special reference to European taxa

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Abstract — Ninety-one *Melanoleuca* collections were chosen to test the agreement of current and traditional infrageneric tripartite classifications of *Melanoleuca* (subgenera *Acystis* without cystidia, *Urticocystis* with urticiform cystidia, and *Melanoleuca* with macrocystidia) with molecular phylogenetic data (ITS sequences analysis) and to evaluate the systematic significance of relevant morphological characters. *Melanoleuca* is found to be monophyletic, and only two emended subgenera, *Urticocystis* and *Melanoleuca*, are supported. Subg. *Urticocystis* comprises all taxa with urticoid cystidia plus the macrocystidiate *M. cognata* complex. The artificial subg. *Acystis* is shown to be polyphyletic and no longer tenable. The entire genus comprises at least 10 clades with 13 subclades. *Melanoleuca sublanipes* sp. nov. and new combinations *M. exscissa* f. *iris*, *M. exscissa* f. *sarcophylla*, *M. exscissa* f. *diverticulata*, and *M. paedida* f. *electropoda* are introduced.

Key words — *Basidiomycota*, *Agaricomycetes*, *Kinia*, pluteoid clade, taxonomy

Introduction

Kirk et al. (2008) state that the basidiomycete genus *Melanoleuca* Pat., typified by *M. vulgaris* (Pat.) Pat. [= *M. melaleuca* (Pers.) Murrill] and traditionally placed in subtribe *Leucopaxillineae* Singer (*Tricholomataceae* R. Heim ex Pouzar, *Agaricales* Underw.) (Singer 1986), comprises approximately 50 saprobic species worldwide. However, Index Fungorum (http://www.indexfungorum.org/, accessed 28 June 2011), lists 332 validly published *Melanoleuca* names representing 126 European and 206 extra-European taxa (pers. obs.). Of the 164 names that actually represent the genus as presently circumscribed (cf. Pfister

1984), many are now known as synonyms with others yet to be listed as such. New taxa are, however, continuously being described, even from well-studied areas such as Europe (e.g., Bresinsky 2006, this paper). *Melanoleuca* species are cosmopolitan and characterised by the following characters: collybioid to tricholomatoid basidiomata; convex to slightly depressed (often with a shallow umbo) pilei; emarginated to adnate to shortly decurrent lamellae; absence of veils; white to pale-yellowish spore print; cutis to trichoderm pileipellis; hyaline spores with amyloid ornamentations; cheilocystidia (mostly present) of two types either urticoid, septate, thin-walled or fusiform to lageniform, mostly aseptate, slightly thick-walled, sometimes with encrusting crystals at apex; pleurocystidia similar to cheilocystidia; no clamp connections (Pegler & Young 1973, Gillman & Miller 1977, Kühner 1978, Singer 1986, Boekhout 1988, 1999; Bon 1991, Vesterholt 2008, Watling & Turnbull 2008). *Leucopaxillus* Boursier, a morphologically allied genus, differs from *Melanoleuca* mainly in abundant clamp connections and (usually) lacking well-developed hymenial cystidia (Singer 1986, Bon 1991). But, according to recent molecular analyses (Moncalvo et al. 2002, Matheny et al. 2006), *Melanoleuca* and *Leucopaxillus* are not closely related: *Melanoleuca* species cluster within the Pluteoid clade (*Pluteaceae* Kotl. & Pouzar), whereas *Leucopaxillus* belongs to the Tricholomatoid clade, close to *Tricholoma* (Fr.) Staude. Sequence analyses by Moncalvo et al. (2000, 2002) place *Melanoleuca* and *Pluteus* Fr. as sister to *Amanitaceae* R. Heim ex Pouzar, while others place the minute uniloculate gasteromycete, *Limnoperdon incarnatum* G.A. Escobar, sister to *Melanoleuca* and *Pluteus* (Bodensteiner et al. 2004; Binder et al. 2006; Vizzini et al. 2010) or to *Melanoleuca*, *Pluteus* and *Volvariella* Speg. (Matheny et al. 2006). Justo et al. (2011) recently place *Melanoleuca* as sister to a monophyletic group formed by *Pluteus* species and *Volvopluteus* Vizzini et al. Finally, Vizzini et al. (2010) reduced the puzzling genus *Kinia* Consiglio et al. to a subgenus of *Melanoleuca* with non-amyloid spores.

Melanoleuca is one of the less appealing fungal genera, whose members are mostly tedious and drab in appearance and dull in pileus colours. It is a character-poor genus with many species macroscopically very similar and differing only in very subtle features (e.g., basidioma colour, odour, stipe ornamentation) and a morphology strongly influenced by environmental factors (Bon 1991, Boekhout 1999). Thus far, infrageneric classifications and species circumscriptions have relied on morphological characters. Identification especially depends on microscopic observations of a rather limited set of characters, such as presence/absence of cheilocystidia, cystidial shape, spore size and ornamentation, and pileipellis structure. Interpretation of some characters may rely on personal experience (e.g. shape of cystidia); in some cases the value ranges overlap (e.g. spore size), in others pileipellis structure and spore

size show great intra-basidiome variability. From a traditional morphological perspective, this often makes species identification difficult or even daunting. The paucity of *Melanoleuca* characters has been detrimental to establishing a natural taxonomic framework for rationalizing infrageneric relationships.

The lack of a modern *Melanoleuca* monograph and the existence of many short, taxonomically and geographically limited publications further complicate the situation. In addition, there are many controversies concerning the interpretations of old descriptions and names (Boekhout 1988).

Several different infrageneric classifications of *Melanoleuca* have been published. Singer (1935, 1943, 1986), Métrod (1942, 1948), Kühner (1978), and Moser (1983) proposed schemes based mainly on macromorphological features (see Boekhout 1988 for an historical review). Singer (1986), for example, divided *Melanoleuca* into four sections —*Alboflavidae* Singer, *Humiles* Singer, *Oreinae* Singer, *Melanoleuca*— circumscribed only by pileus colour and stipe ornamentation.

Bon (1978), the first to present an infrageneric classification based equally on macro- and micro-morphological characters, divided *Melanoleuca* into seven sections. Boekhout (1988), focusing mainly on microscopic features, stressed the importance of absence/presence and shape of cystidia (first noted by Metrod 1948) and divided the genus into three subgenera (Tab. 1): subg. *Melanoleuca*— no cystidia; subg. *Urticocystis* Boekhout—with urticiform cheilocystidia and no (or very rare) pleurocystidia; subg. *Macrocystis* Boekhout—with long fusiform to lageniform cheilocystidia (macrocystidia) and similar pleurocystidia. Boekhout recognized two urticiform types: the *brevipes*-type (with narrow cylindrical upper part, Fig. 1a) and the *exscissa*-type (with rather wide upper part attenuating towards the apex, Fig. 1b).

Bon (1991) proposed a slightly different infrageneric classification (Tab. 1) by introducing the spore Q value (the ratio of length to width of the spores in side view) and stressing the importance of fusiform versus lageniform cystidia (Fig. 1c–d) as important key characters for delimiting subsections.

Finally, Boekhout (1999) reintroduced a simplified classification practically identical to the 1988 scheme except that sect. *Grammopodiae* was no longer subdivided into subsects. *Grammopodiae* and *Exscissae* (Tab. 1).

There are only two major monographic treatments of *Melanoleuca*: Bon (1991) covers over 80 taxa, most reported only from Europe, and Boekhout (1999), covers only Dutch taxa and unites species into large complexes thereby recognizing only 14 species and several forms and varieties.

Bon (1991), who covered a larger number of taxa, was used both for selecting which species to sample and for testing the effectiveness of Bon's classification method. Due to the limitation of morphological characters used, several of his taxonomical units remain controversial.

Table 1. The tripartite infrageneric classifications of *Melanoleuca* by Bon and Boekhout.

Boekhout's classification (1988, 1999)
Subg. *Melanoleuca* – without cystidia.
Subg. *Urticocystis* – cheilocystidia urticiform and pleurocystidia absent or very rare.
Sect. *Humiles* – stipe squamulose or verrucose throughout; stipe usually much longer than diameter of the pileus.
Sect. *Grammopodiae* – stipe smooth or somewhat fibrillose; stipe much shorter than or equally large as diameter of pileus.
*Subsect. *Grammopodiae* – urticiform cystidia of the *brevipes*-type.
*Subsect. *Exscissae* – urticiform cystidia of the *exscissa*-type.
Subg. *Macrocystis* – cheilocystidia fusiform to lageniform and pleurocystidia similar.
Sect. *Cognatae* – with a bright coloured pileus.
Sect. *Alboflavidae* – with a white to cream-whitish pileus.
Sect. *Strictipedes* – with a grey-brown pileus.
Bon' s classification (1991)
Subg. *Acystis* – species without cystidia.
Sect. *Acystis* – spores with a Q<1.4(–1.5).
Sect. *Decembres* – spores with a Q>(1.5–) 1.6.
Subg. *Urticocystis* – cheilocystidia urticiform to strictly lageniform, septate, up to 50(–60) µm long.
Sect. *Humiles* – stipe squamulose to dark-dotted.
Sect. *Grammopodiae* – stipe smooth or striate, sometimes pruinose or flocky.
Subsect. *Rasilinae* – spores with a Q<1.4(–1.5), with isolated warts, cystidia typically urticiform.
Subsect. *Grammopodiae* – spores with a Q>(1.5–)1.6, cystidia typically urticiform.
Subsect. *Exscissae* – spores with a Q>(1.5–)1.6, urticiform cystidia of the *exscissa*-type.
Subg. *Melanoleuca* – macrocystidia fusiform to lageniform, non-septate, up to (40–)50–90(–110) µm long.
Sect. *Alboflavidae* – basidiomes white to whitish.
Sect. *Cognatae* – basidiomes with bright colours, pileus and lamellae concolorous.
Sect. *Oreinae* – basidiomes small (<4(–6) cm), collybioid, often dark coloured.
Sect. *Melanoleuca* – basidiomes small to medium-sized (>(5–) 6 cm), with variable colourations.
Subsect. *Strictipedinae* – cystidia mainly lageniform.
Subsect. *Vulgarinae* – cystidia mainly fusiform.

* = subsections present in the 1988 classification scheme only.

The present study is based on a large ITS sequence dataset and is the first to examine *Melanoleuca* extensively. Our aims were to 1) check whether *Melanoleuca* is monophyletic as traditionally circumscribed; 2) test Bon's (1991) morphologically based taxonomy against molecular phylogenetic data; 3) evaluate whether traditional or other morphological features (e.g., pileus colour, spore and cheilocystidia shape) reflect phylogenetic relationships.

Materials & methods

Taxon sampling

Samples from 91 *Melanoleuca* collections (41 taxa, 18 unidentified *Melanoleuca* sp.; Tab. 2) were tested. Specimens were collected fresh, dried, and deposited in ANC (Erbario

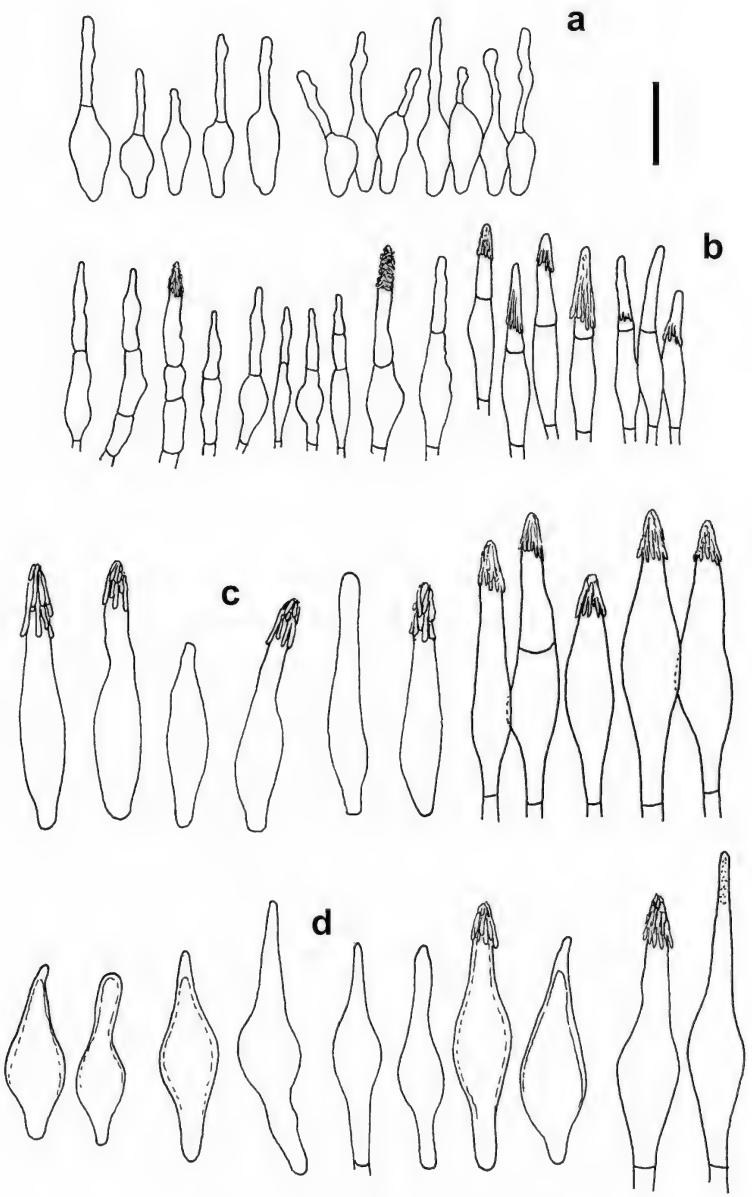

FIGURE 1. Types of cystidia in *Melanoleuca*.
a–b. Urticiform cystidia (a = *brevipes*-type; b = *exscissa*-type).
c–d. Macrocystidia (c = fusiform; d = lageniform).
Bar = 20 μm.

Table 2. *Melanoleuca* collections examined (species with multiple collections numbered consecutively; see also Fig. 2).

Collections	Coll. ID./Origin	Subgenera / Sections (Bon 1991)	ITS Acc. No.
* *Melanoleuca albifolia* 1	ANC M0182/Spain	*Melanoleuca / Oreinae*	JN616418
* *M. albifolia* 2	ANC M0184/Italy	*Melanoleuca / Oreinae*	JN616419
* *M. angelesiana* 1	ANC M0203/Italy	*Acystis / Acystis*	JN616420
* *M. angelesiana* 2	ANC M0204/Italy	*Acystis / Acystis*	JN616421
* *M. arcuata*	ANC M0167/Italy	*Melanoleuca / Cognatae*	JN616422
* *M. atripes*	ANC M0180/Italy	*Melanoleuca / Oreinae*	JN616423
* *M. bataillei*	ANC M0185/Italy	*Melanoleuca / Melanoleuca*	JN616424
M. brevipes 1	MCVE 04574/Italy	*Urticocystis /Grammopodiae*	JF908352
M. brevipes 2	MCVE 04505/Italy	*Urticocystis /Grammopodiae*	JF908351
M. cinereifolia 1	MCVE 01471/Italy	*Melanoleuca / Melanoleuca*	JN052138
M. cinereifolia 2	MCVE 11243/Italy	*Melanoleuca / Melanoleuca*	JF908356
M. cinereifolia 3	MCVE 20748/Italy	*Melanoleuca / Melanoleuca*	JN052137
M. cognata 1	MCVE 13939/Italy	*Melanoleuca / Cognatae*	JF908360
* *M. cognata* 2	ANC M0170/Italy	*Melanoleuca / Cognatae*	JN616425
* *M. decembris* 1	ANC M0197/Italy	*Acystis / Decembres*	JN616426
* *M. decembris* 2	ANC M0199/Italy	*Acystis / Decembres*	JN616427
* *M. decembris* 3	ANC M0200/Italy	*Acystis / Decembres*	JN616428
M. decembris 4	MCVE 01573/Italy	*Acystis / Decembres*	JF908346
* *M. diverticulata*	ANC M0206/Italy	*Urticocystis /Grammopodiae*	JN616429
* *M. electropoda*	ANC M0187/Italy	*Melanoleuca / Oreinae*	JN616430
M. evenosa	MCVE 14576/Italy	*Melanoleuca / Alboflavidae*	JN052142
* *M. exscissa* 1	ANC M0207/Italy	*Urticocystis /Grammopodiae*	JN616431
* *M. exscissa* 2	ANC M0208/Italy	*Urticocystis /Grammopodiae*	JN616432
* *M. exscissa* 3	ANC M0210/Italy	*Urticocystis /Grammopodiae*	JN616433
* *M. exscissa* 4	ANC M0210B/Italy	*Urticocystis /Grammopodiae*	JN616434
* *M. exscissa* 5	ANC M0212/Italy	*Urticocystis /Grammopodiae*	JN616435
* *M. exscissa* 6	ANC M0213/Italy	*Urticocystis /Grammopodiae*	JN616436
* *M. friesii*	ANC M0186/Italy	*Melanoleuca / Melanoleuca*	JN616437
* *M. graminicola*	ANC M0201/Italy	*Acystis / Acystis*	JN616438
* *M. grammopodia* 1	ANC M0217/Italy	*Urticocystis /Grammopodiae*	JN616439
* *M. grammopodia* 2	ANC M0218/Italy	*Urticocystis /Grammopodiae*	JN616440
* *M. grammopodia* 3	ANC M0219/Slovenia	*Urticocystis /Grammopodiae*	JN616441
* *M. grammopodia* f. *macrocarpa* 1	ANC M0215/Italy	*Urticocystis /Grammopodiae*	JN616442
* *M. grammopodia* f. *macrocarpa* 2	ANC M0216/Italy	*Urticocystis /Grammopodiae*	JN616443
M. grammopodia f. *macrocarpa* 3	MCVE 04410/Italy	*Urticocystis /Grammopodiae*	JF908350
* *M. heterocystidiosa* 1	ANC M0174/Italy	*Melanoleuca / Melanoleuca*	JN616444
* *M. heterocystidiosa* 2	ANC M0175/Italy	*Melanoleuca / Melanoleuca*	JN616445
* *M. iris*	ANC M0211/Italy	*Urticocystis /Grammopodiae*	JN616446
* *M. "lanipes"*	ANC M0166/Italy	*Melanoleuca / Melanoleuca*	JN616447
* *M. melaleuca*	ANC M0176/Italy	*Melanoleuca / Melanoleuca*	JN616448
* *M. microcephala*	ANC M0196/Italy	*Urticocystis /Grammopodiae*	JN616449
* *M. nivea* 1	ANC M0177/Italy	*Melanoleuca / Alboflavidae*	JN616450
* *M. nivea* 2	ANC M0183/Italy	*Melanoleuca / Alboflavidae*	JN616451
M. nivea 3	MCVE 09578/Italy	*Melanoleuca / Alboflavidae*	JN392452

* = collections newly sequenced in this study.

Table 2, concluded

Collections	Coll. ID./Origin	Subgenera / Sections (Bon 1991)	ITS Acc. No.
M. oreina	MCVE 07839/Italy	*Melanoleuca / Oreinae*	JN392450
* *M. paedida* 1	ANC M0189/Italy	*Urticocystis /Grammopodiae*	JN616452
* *M. paedida* 2	ANC M0190/Italy	*Urticocystis /Grammopodiae*	JN616453
M. "paratristis"	MCVE 12645/Italy	*Acystis / Acystis*	JF908357
* *M. privernensis*	GC 08310 holotype/Italy	Subgen. *Kinia* (Vizzini et al. 2010)	JN616454
* *M. pseudoluscina* 1	ANC M0191/Italy	*Urticocystis /Grammopodiae*	JN616455
* *M. pseudoluscina* 2	ANC M0192/Italy	*Urticocystis /Grammopodiae*	JN616456
* *M. pseudoluscina* 3	ANC M0193/Italy	*Urticocystis /Grammopodiae*	JN616457
* *M. pseudoluscina* 4	ANC M0194/Italy	*Urticocystis /Grammopodiae*	JN616458
* *M. pseudoluscina* 5	ANC M0195/Italy	*Urticocystis /Grammopodiae*	JN616459
* *M. pseudopaedida*	ANC M0198/Italy	*Acystis / Acystis*	JN616460
* *M. rasilis*	ANC M0220/Italy	*Urticocystis /Grammopodiae*	JN616461
* *M. robertiana*	ANC M0205/Italy	*Acystis / Acystis*	JN616462
* *M. robusta*	ANC M0179/Italy	*Melanoleuca / Melanoleuca*	JN616463
* *M. strictipes* 1	ANC M0171/Italy	*Melanoleuca / Alboflavidae*	JN616464
* *M. strictipes* 2	ANC M0172/Italy	*Melanoleuca / Alboflavidae*	JN616465
* *M. strictipes* 3	ANC M0173/Italy	*Melanoleuca / Alboflavidae*	JN616466
* *M. stridula*	ANC M0007/Italy	*Acystis / Acystis*	JN616467
* *M. striimarginata*	ANC M0202/Italy	*Acystis / Acystis*	JN616468
M. subalpina	MCVE 04112/Italy	*Melanoleuca / Alboflavidae*	JN052139
* *M. sublanipes* 1	ANC M0221/Italy	*Urticocystis /Grammopodiae*	JN616469
* *M. sublanipes* 2	ANC M0222 holotype/Italy	*Urticocystis /Grammopodiae*	JN616470
* *M. sublanipes* 3	ANC M0223/France	*Urticocystis /Grammopodiae*	JN616471
* *M. subpulverulenta* 1	ANC M0004/Italy	*Melanoleuca / Melanoleuca*	JN616472
* *M. subpulverulenta* 2	ANC M0178/Italy	*Melanoleuca / Melanoleuca*	JN616473
* *M. substrictipes* 1	ANC M0214/Italy	*Urticocystis /Grammopodiae*	JN616474
M. substrictipes 2	MCVE 13934/Italy	*Urticocystis /Grammopodiae*	JF908359
* *M. substrictipes* var. *sarcophylla*	ANC M0209/Italy	*Urticocystis /Grammopodiae*	JN616475
M. verrucipes	MCVE 09962Switzerland	*Urticocystis / Humiles*	JF908354
* *Melanoleuca* sp. 1	ANC M0181/Italy	*Acystis / Acystis*	JN616476
* *M.* sp. 2	ANC M0188/Italy	*Acystis / Acystis*	JN616477
* *M.* sp. 3	ANC M0224/Italy	*Melanoleuca / Melanoleuca*	JN616478
M. sp. 4	MCVE 12248/Italy	*Melanoleuca / Melanoleuca*	JN052141
M. sp. 5	MCVE 13410/Italy	*Melanoleuca / Melanoleuca*	JF908358
M. sp. 6	MCVE 09821/Italy	*Melanoleuca / Melanoleuca*	JN392453
M. sp. 7	MCVE 01687/Italy	*Melanoleuca / Melanoleuca*	JN392449
M. sp. 8	MCVE 01683/Italy	*Melanoleuca / Melanoleuca*	JN392448
M. sp. 9	MCVE 19223/Italy	*Melanoleuca / Melanoleuca*	JN392446
M. sp. 10	MCVE 08389/Italy	*Melanoleuca / Cognatae*	JF908353
M. sp. 11	MCVE 08432/Italy	*Melanoleuca / Melanoleuca*	JN052140
M. sp. 12	MCVE 14221/Italy	*Urticocystis /Grammopodiae*	JN392454
M. sp. 13	MCVE 08384/Italy	*Urticocystis /Grammopodiae*	JN392451
M. sp. 14	MCVE 03316/Italy	*Urticocystis /Grammopodiae*	JF908349
M. sp. 15	MCVE 19627/Italy	*Urticocystis /Grammopodiae*	JF908344
* *M.* sp. 16	MCVE 24095/Italy	*Urticocystis /Grammopodiae*	JN616479
M. sp. 17	MCVE 01681/Italy	*Urticocystis /Grammopodiae*	JN392447
M. sp. 18	MCVE 14273/Italy	*Acystis / Decembres*	JF908362

dell'Università di Ancona, Italy) or acquired from MCVE (the Museo di Storia Naturale di Venezia Herbarium, Italy) (Tab. 2). MCVE collections were chosen, even where often misdetermined, because DNA had already been bar-coded by Dr. M. Garbelotto (University of California, Berkeley). All *Melanoleuca* collections were identified or redetermined using i) an unpublished key based on the protologues or referenced to original collections (Fontenla & Para, ined.) and ii) existing monographs (Bon 1991, Boekhout 1999, Fontenla et al. 2003). Watling & Turnbull (1983), Horak (2005), and Vesterholt (2008) were also consulted. When not identifiable, collections are cited in Tab. 2 and Fig. 2 as *Melanoleuca* sp. *Melanoleuca* species were selected to represent subgenera and sections in Bon (1991; Tab. 1). Each section was represented by at least two species except for sect. *Humiles* (represented only by *M. verrucipes*). *Melanoleuca privernensis* (Consiglio et al.) Consiglio et al. [≡ *Kinia privernensis*] was also included in the dataset in accordance with Vizzini et al. (2010). In the species descriptions Q = quotient of length and width of the spores in side view and Qm = average quotient. The spore Qm value and cystidia shape are reported for each collection in Fig. 2. Herbarium abbreviations follow Thiers (2011). Author citations follow the Index Fungorum-Authors of Fungal Names (http://www.indexfungorum.org/authorsoffungalnames.htm) and the names of new taxa are deposited in MycoBank (http://www.mycobank.org/DefaultPage.aspx).

DNA extraction, PCR amplification, DNA sequencing

Genomic DNA was isolated, extracted from 62 dried herbarium specimens (Tab. 2) using the DNeasy Plant Mini Kit (QIAGEN, Milan, Italy). Universal primers ITS1F/ITS4 were used for ITS region amplification (White et al. 1990, Gardes & Bruns 1993). Amplification reactions were performed in a PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems) in 25 mL reaction mixtures with these final concentrations or total amounts: 5 ng DNA, 13 PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 1 mM each primer, 2.5mM MgCl2, 0.25mM each dNTP, 0.5 unit Taq polymerase (Promega). The PCR program was 3 min at 95 C for one cycle, 30 s at 94 C, 45 s at 50 C, 2 min at 72 C for 35 cycles, 10 min at 72 C for one cycle. PCR products were resolved on a 1.0%agarose gel and visualized by staining with ethidium bromide. PCR products were purified with the AMPure XP kit (Beckman) and sequenced by DiNAMYCODE srl (Turin, Italy). Sequences were assembled and edited with the phred/phrap/consed software suite and submitted to GenBank (Tab. 2).

Sequence alignment and phylogenetic analysis

Sequences included in the phylogenetic analyses were generated in this study (Tab. 2) or retrieved from GenBank. GenBank sequences were selected based on other phylogenetic studies on *Agaricales* (Moncalvo et al. 2002; Matheny et al. 2006; Justo et al. 2011). *Limnoperdon incarnatum* (DQ097363) was used as outgroup taxon. Other taxa belonging to the Pluteoid clade were included in the analysis for testing the monophyly of *Melanoleuca*.

Sequences obtained in this study were checked and assembled using Geneious v5.3 (Drummond et al. 2010). Alignment of the ITS dataset was generated using MAFFT v6.814b (Katoh et al. 2002) with default conditions for gap openings and gap extension penalties. The alignment was then imported into MEGA 5.0 (Tamura et al. 2011) for

manual adjustment. Best-fit models were estimated by both the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) using jModelTest 0.1.1 (Posada 2008) to provide a substitution model for each single alignment. TPM2uf+I+G model was chosen. Phylogenetic hypotheses were constructed under Bayesian Inference (BI) and Maximum Likelihood (ML) criteria.

BI of phylogeny using Monte Carlo Markov Chains (MCMC) was carried out with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). Four incrementally heated simultaneous MCMC were run over 10.000.000 generations, under model assumption. Trees were sampled every 1.000 generations resulting in an overall sampling of 10.001 trees. The "burn-in" value was evaluated using Tracer 1.5 (Rambaut & Drummond 2007). The first 15% of trees was discarded as "burn-in". For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian Posterior Probabilities (BPP). Branch lengths were estimated as mean values over the sampled trees. Only BPP values over 50% are reported in the resulting trees. This Bayesian analysis was repeated three times, always using random starting trees and random starting values for model parameters to test the independence of the results from the revisiting of the prior topologies during chain growth (Huelsenbeck et al. 2002).

ML estimation was performed through RAxML v.7.0.4 (Stamatakis 2006) with 1,000 bootstrap replicates (Felsenstein 1985) using the GTRGAMMAI algorithm to perform a tree inference and search for a good topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the "-f a" option of RAxML and "-x 12345" as a random seed to invoke the novel rapid bootstrapping algorithm. Support values for major clades that are supported by either BI and ML analyses are visualized in the resulting tree.

Analysis of the pairwise % identity values (hereafter shortened as P%IV) for the *Melanoleuca* sequences were calculated using MEGA 5.0 (Tamura et al. 2011).

Results

Our phylogenetic results are presented in Fig. 2. The general ITS data matrix comprises a total of 105 sequences (including 14 from GenBank). This 898 bp dataset contains 621 (69.2%) variable sites, of which 525 (58.4%) are parsimony-informative. The Bayesian and ML tree topologies are congruent. Our analyses show that *Melanoleuca* is clearly monophyletic (1.0 BPP and 89% MLB). Two major clades, A and B, were distinguished within *Melanoleuca*. Clade A is supported by only BI tree with 0.79 BPP, while clade B is well supported with 1.0 BPP and 99% MLB. Clade A consists of 5 clades (A1–A5, with 10 subclades) and clade B of 5 clades (B1–B5, with 3 subclades).

Discussion

Melanoleuca and infrageneric classification

The Bayesian (1.0 BPP) and ML (89% MLB) analyses strongly support *Melanoleuca* as monophyletic and distributed throughout the ingroup within

2 major clades (A and B) with 10 smaller clades (A1–A5 and B1–B5) (Fig. 2). The two sequences of *M. subsejuncta* (Peck) Murrill from GenBank (FJ596898; FJ596898) cluster outside *Melanoleuca*, sister to *Amanitaceae*; Pfister (1984), who examined its type, referred the collection to *Tricholoma*. Results of our phylogenetic analyses and the current morphology-based infrageneric classification of *Melanoleuca* are not congruent. In particular, our data (Fig. 2) are incompatible with the tripartite infrageneric *Melanoleuca* classification of Bon (1991) and Boekhout (1988, 1999) (Tab. 1). Based on molecular data, species traditionally ascribed to subgenus *Acystis* Bon (= subgenus *Melanoleuca* sensu Boekhout 1988, 1999) do not form a monophyletic assemblage and are distributed over the *Melanoleuca* clade (clade A and B, Fig. 2).

The fact that acystidiate taxa are not phylogenetically related implies that cystidial acquisition and loss have taken place independently during the evolution of *Melanoleuca* and also that this character is homoplastic and unsuitable for a natural classification of these fungi. Subgenus *Acystis* should therefore be considered an artificial superfluous taxon that is no longer tenable. In addition, in some cases (e.g. subclades A4.1 & A4.2; clade B2, Fig. 2), cystidiate and non-cystidiate taxa are conspecific (syntaxic): the acystidiate *M.* sp. 18, *M.* sp. 2, and *M.* sp. 1 represent only forms of the cystidiate taxa *M. pseudoluscina* Bon, *M. paedida* (Fr.) Kühner & Maire, and *M. atripes* Boekhout/*M. albifolia* Boekhout, respectively. It is conceivable that the shift from cystidiate to non-cystidiate basidiomata and vice versa within a single species is controlled by a limited number of genes that are switched off by so far unknown environmental factors.

Most macrocystidiate taxa in subg. *Melanoleuca* (= *Macrocystis* Boekhout) form clade B (Fig. 2), while the *M. cognata* complex (A5 clade, = sect. *Cognatae*) nests with all species in subg. *Urticocystis* to form clade A (Fig. 2). Therefore, subg. *Melanoleuca* as traditionally circumscribed (Bon 1991) is polyphyletic and subg. *Urticocystis* is paraphyletic when sect. *Cognatae* is excluded. It would appear that even cystidial shape is not a reliable character for tracing higher phylogenetic relationships.

Our analysis therefore implies that only two subgenera (clades A and B) should be recognized in *Melanoleuca*:

Melanoleuca Pat. subg. ***Melanoleuca***, emend. Fontenla, Para & Vizzini

Clade B (the autonymous subgenus, including *M. melaleuca*), characterized by basidiomata with non-septate macrocystidia, or rarely without cystidia.

Melanoleuca subg. ***Urticocystis*** Boekhout, Persoonia 13(4): 400 (1988), emend. Fontenla, Para & Vizzini

Clade A (type species: *M. grammopodia* (Bull.) Murrill), comprising taxa mainly with urticocystidia but also with macrocystidia and brightly coloured pilei (sect. *Cognatae*), or lacking cystidia.

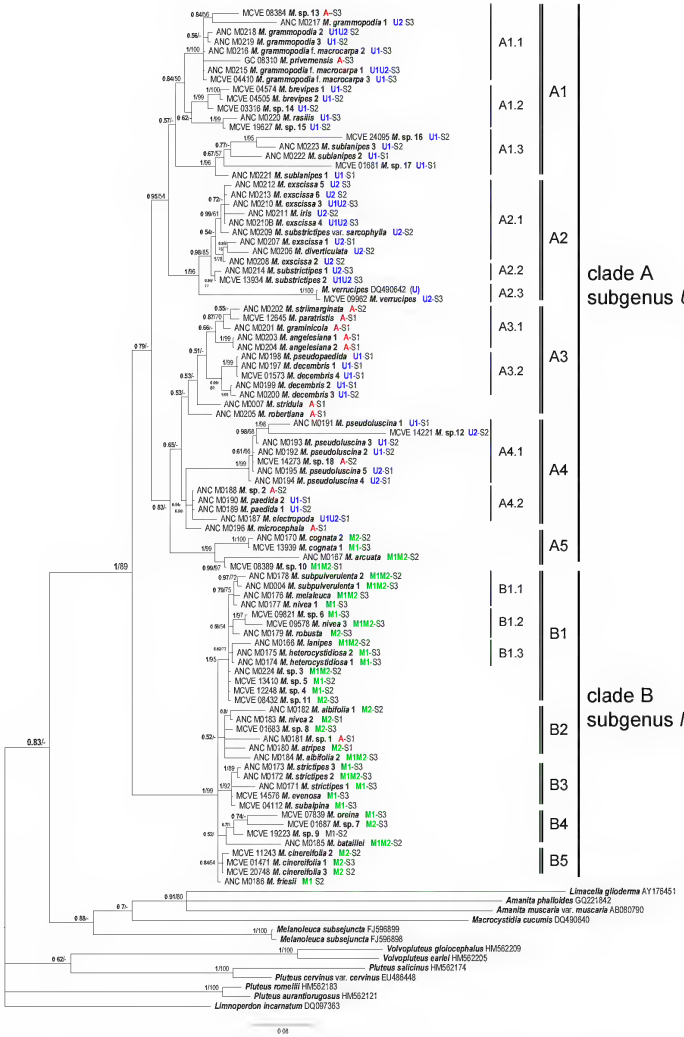
A1.1
A1.2
A1.3
A1
A2.1
A2.2
A2.3
A2
clade A
subgenus Urticocystis
A3.1
A3.2
A3
A4.1
A4.2
A4
A5
B1.1
B1.2
B1.3
B1
B2
B3
B4
B5
clade B
subgenus Melanoleuca
MCVE 08384 M. sp. 13 A-S3
ANC M0217 M. grammopodia 1 U2 S3
ANC M0218 M. grammopodia 2 U1U2 S2
ANC M0219 M. grammopodia 3 U1 S2
ANC M0216 M. grammopodia f. macrocarpa 2 U1-S3
GC 08310 M. privemensis A-S3
ANC M0215 M. grammopodia f. macrocarpa 1 U1U2-S3
MCVE 04410 M. grammopodia f. macrocarpa 3 U1-S3
MCVE 04574 M. brevipes 1 U1-S2
MCVE 04505 M. brevipes 2 U1-S2
MCVE 03316 M. sp. 14 U1-S2
ANC M0220 M. rasilis U1-S3
MCVE 19627 M. sp. 15 U1 S2
MCVE 24085 M. sp. 16 U1-S2
ANC M0223 M. sublanipes 3 U1-S2
ANC M0222 M. sublanipes 2 U1-S1
MCVE 01681 M. sp. 17 U1-S1
ANC M0221 M. sublanipes 1 U1 S1
ANC M0212 M. exscissa 5 U2 S3
ANC M0213 M. exscissa 6 U2 S2
ANC M0210 M. exscissa 3 U1U2 S3
ANC M0211 M. iris U2-S2
ANC M0210B M. exscissa 4 U1U2 S3
ANC M0209 M. substrictipes var. sarcophylla U2-S2
ANC M0207 M. exscissa 1 U2 S1
ANC M0205 M. diverticulata U2 S2
ANC M0208 M. exscissa 2 U2 S2
ANC M0214 M. substrictipes 1 U2-S3
MCVE 13934 M. substrictipes 2 U1U2 S3
M. verrucipes DQ490642 (U)
MCVE 05962 M. verrucipes U2-S3
ANC M0202 M. striimarginata A-S2
MCVE 12645 M. parastrictis A-S1
ANC M0201 M. graminicola A-S1
ANC M0203 M. angelesiana 1 A-S1
ANC M0204 M. angelesiana 2 A-S1
ANC M0198 M. pseudopaedida U1 S1
ANC M0197 M. decembris 1 U1-S1
MCVE 01573 M. decembris 4 U1-S1
ANC M0199 M. decembris 2 U1-S1
ANC M0200 M. decembris 3 U1-S2
ANC M0007 M. stridula A S1
ANC M0205 M. robertiana A-S1
ANC M0191 M. pseudoluscina 1 U1-S1
MCVE 14221 M. sp.12 U2-S2
ANC M0193 M. pseudoluscina 3 U1-S2
ANC M0192 M. pseudoluscina 2 U1-S2
MCVE 14273 M. sp. 18 A-S2
ANC M0195 M. pseudoluscina 5 U2-S1
ANC M0194 M. pseudoluscina 4 U2-S1
ANC M0188 M. sp. 2 A-S2
ANC M0190 M. paedida 2 U1-S1
ANC M0189 M. paedida 1 U1-S2
ANC M0187 M. electropoda U1U2-S1
ANC M0196 M. microcephala A-S1
ANC M0170 M. cognata 2 M2-S2
MCVE 13939 M. cognata 1 M1-S3
ANC M0167 M. arcuata M1M2-S2
MCVE 08389 M. sp. 10 M1M2-S1
ANC M0178 M. subpulverulenta 2 M1M2-S2
ANC M0004 M. subpulverulenta 1 M1M2-S3
ANC M0176 M. melaleuca M1M2 S3
ANC M0177 M. nivea 1 M1 S3
MCVE 09821 M. sp. 6 M1-S3
MCVE 09578 M. nivea 3 M1M2-S3
ANC M0179 M. robusta M2-S3
ANC M0168 M. lenipes M1M2-S2
ANC M0175 M. heterocystidiosa 2 M1-S3
ANC M0174 M. heterocystidiosa 1 M1-S3
ANC M0224 M. sp. 3 M1M2-S2
MCVE 13410 M. sp. 5 M1-S2
MCVE 12248 M. sp. 4 M1-S2
MCVE 08432 M. sp. 11 M2-S3
ANC M0182 M. albifolia 1 M2-S2
ANC M0183 M. nivea 2 M2-S1
MCVE 01683 M. sp. 8 M2-S3
ANC M0181 M. sp. 1 A-S1
ANC M0180 M. stripes M2-S1
ANC M0184 M. albifolia 2 M1M2 S3
ANC M0173 M. strictipes 3 M1-S3
ANC M0172 M. strictipes 2 M1M2-S3
ANC M0171 M. strictipes 1 M1-S3
MCVE 14576 M. evenosa M1-S3
MCVE 04112 M. subalpina M1-S3
MCVE 07839 M. oreina M1-S3
MCVE 01687 M. sp. 7 M2-S3
MCVE 19223 M. sp. 9 M1-S2
ANC M0185 M. batalliei M1M2-S2
MCVE 11243 M. cinereifolia 2 M2-S2
MCVE 01471 M. cinereifolia 1 M2-S3
MCVE 20748 M. cinereifolia 3 M2 S2
ANC M0186 M. friesii M1 S2
Limacella glioderma AY176451
Amanita phalloides GQ221842
Amanita muscaria var. muscaria AB080790
Macrocystidia cucumis DQ490640
Melanoleuca subsejuncta FJ596899
Melanoleuca subsejuncta FJ596898
Volvopluteus gloiocephalus HM562209
Volvopluteus earlei HM562205
Pluteus salicinus HM562174
Pluteus cervinus var. cervinus EU486448
Pluteus romellii HM562183
Pluteus aurantiorugosus HM562121
Limnoperdon incarnatum DQ097363
0.08

FIGURE 2. Bayesian phylogram obtained from the ITS rDNA sequences of *Melanoleuca* and other taxa of the Pluteoid clade. *Limnoperdon incarnatum* was used as outgroup taxon. Support values (**BPP** in bold >0.5; MLB >50%) are given above branches. Minor supported clades discussed in the text are numbered A1–A5 and B1–B5. Numbers refer to the collections cited in TAB. 2.

Characters: 1) presence/absence of cheilocystidia and type of cheilocystidia — **A** = taxa without cheilocystidia, **U1** = taxa with *brevipes*-type urticiform cystidia, **U2** = taxa with *exscissa*-type urticiform cystidia, **U1-U2** = taxa with both types of urticiform cystidia, **M1** = taxa with fusiform macrocystidia, **M2** = taxa with lageniform macrocystidia, **M1-M2** = taxa with both type of macrocystidia [cystidial types are coded in red (A), blue (U), and green (M)]; 2) spore shape (Qm range) – **S1** = Qm < 1.40; **S2** = 1.40≤ Qm ≤1.60; **S3** = Qm > 1.60..

Basidiospore shape (Qm value) seems to be homoplastic, too variable to use for circumscribing sections and subsections as proposed by Bon (1991). For example, in subclade A2.1, collections otherwise assignable to *M. exscissa* (Fr.) Singer show a great Qm range, as do *M. grammopodia* (A1.1) and *M. pseudoluscina* (A4.1) collections (Fig. 2).

On the contrary, cystidial subtypes may have some value for delimiting small clades (Fig. 2). Clades A1 and A3 are formed mainly by taxa with *brevipes*-type urticocystidia and clade A2 by taxa by mainly *exscissa*-type urticocystidia. Clade B1 comprises taxa with mainly fusiform macrocystidia.

Pileus colour is not a reliable phylogenetic marker at any taxonomic level: three collections (ANC M0177, MCVE 09578, ANC M0183) determined as *M. nivea* Métrod ex Boekhout, a species traditionally included in sect. *Alboflavidae* based on whitish colouration and presence of macrocystidia (Bon 1991, Boekhout 1988, 1999; Deschuyteneer 2008), are not placed with other species of that section (clade B3) but distributed throughout clade B. The first collection represents an albinic form of *M. melaleuca* (B1.1), the second a taxon close to *M. robusta* (Bres.) Fontenla et al. (B1.2), and the third probably an albinic form of *M. albifolia* (B2).

In conclusion, taxonomically important morphological characters in *Melanoleuca* show a high degree of homoplasy. Although these characters are useful for species delimitation, and in some cases for the circumscription of sections or groups, they appear insufficient for a phylogenetically correct infrageneric concept. A clearer picture will emerge as more *Melanoleuca* diversity is included in future analyses.

Groupings and species limits

More extensive sampling of *Melanoleuca* is needed for a revision of the whole genus. Additional ITS sequencing is necessary to clarify species limits and names for taxa occurring in both Europe and North America. For the time being we only make minor comments on some of the infrageneric clades that were recovered. These numbered clades and subclades are marked in Fig. 2.

Subgenus *Urticocystis* (Clade A): clades A1-A5

Clade A1 (0.57 BPP, / MLB), cheilocystidia mainly of the *brevipes*-type or rarely absent. Stipe striate longitudinally or not.

Subclade A1.1 (1.0 BPP, 100% MLB) (= *M. grammopodia* complex = subsect. *Grammopodiae*). This well supported subclade comprises *M. grammopodia* and *M. grammopodia* f. *macrocarpa* Boekhout, all with urticocystidia, and *M. privernensis* and *M.* sp. 13 without cystidia. All taxa have a distinctly longitudinally striate stipe with a pruinose apex. The eight sequences display a 96.8 P%IV. *Melanoleuca grammopodia* f. *macrocarpa* differs from the type

only by a stipe that is much shorter than pileus diameter (P%IV = 99.4) and so should be considered only a growth form of *M. grammopodia*. *Melanoleuca privernensis* is diagnosed by non-amyloid spores, unique in *Melanoleuca* (Vizzini et al. 2010). The molecular analysis suggests it might represent an aberrant neotenic form of *M. grammopodia* lacking cystidia with non-amyloid spores.

Subclade A1.2 (0.62 BPP, / MLB) includes *M. brevipes* (Bull.) Pat., *M. rasilis* (Fr.) Singer, and two unidentified species (*M.* sp. 14, *M.* sp. 15). *Melanoleuca rasilis* is related to *M. brevipes* based on similar cystidia, grey pileus and stipe colours, and a non-longitudinally striate stipe but differs by smaller basidiomes, a stipe length equal to the pileus diameter, and broadly ellipsoid coarsely ornamented spores (Boekhout 1988, 1999). We also observed that *M. rasilis* has a uniformly white context while *M. brevipes* has a white pileus context and brown stipe context. Molecularly, the two species are clearly different (P%IV = 94.5; Fig. 2).

Subclade A1.3 (1.0 BPP, 96% MLB) comprises *M. sublanipes*, *M.* sp. 16, and *M.* sp. 17. *Melanoleuca sublanipes* (formally described below) is characterized by a tomentose-woolly pileus margin and stipe base. The two *M.* sp. could be conspecific with *M. sublanipes*, but their collections lack of macromorphological data and descriptions. We here propose the following new diagnosis:

Melanoleuca sublanipes Fontenla, Para & Vizzini, **sp. nov.**

MycoBank MB 563140

Pileus -10 cm latus, velutinus, obscure griseo-brunneus, ochraceo-brunneus, griseo-brunneus, a margine pallescens. Lamellae albae, albidae vel pallide griseo-brunneae. Stipes 1.5–7 × 0.3–1.1 cm, lanatus deinde solum inferne lanato-flocculosus, in senectute omnino pruinoso-flocculosus, argenteus, a basi griseo-brunneus, cum ripercussis cyaneis. Caro in pileo albida, in stipite brunnea; odor herbaceus; sapor mitis. Sporae 6.2–9.6 × 3.8–6.6 µm; in medio 7.56 × 5.54 µm; Q = 1.04–1.70; Qm = 1.36, ellipsoideae, conspicuis verrucis singulis, globulosis et amyloideis exornatae. Basidia tetraspora. Cheilocystidia numerosa, 31–55 × 5–8 µm, pili urticae revocantibus, raro fusoidea. Pilei cutis ad marginem ex hyphis erectis, 7–8 µm latis ad instar trichodermatis efformata; hyphae terminales cylindricae, usque ad 100 µm longae. Stipitis cutis pilis clavatis aggregatis et caulocystidiis multiformibus ornata. Habitat in herbidis locis.

Type: Italy, Veneto, Padova, Montegrotto Terme, 07.XI.2008, leg. G. Zecchin (**holotype** ANC M0222).

Etymology: the specific epithet refers to the tomentose-woolly surface of the stipe base.

Clade A2 (1.0 BPP, 96% MLB), cheilocystidia mainly of the *exscissa*-type: subclades A2.1, A2.2 and A2.3.

Subclade A2.1 (0.54 BPP, / MLB) (*M. exscissa* complex) comprises *M. exscissa*, *M. diverticulata*, *M. iris*, and *M. substrictipes* var. *sarcophylla*. The nine sequences display a 98.5 P%IV. This clade reflects an overemphasis on basidiome colour

and smell, with some anatomical traits having led to the establishment of some superfluous species. *Melanoleuca iris* differs from *M. exscissa* only in the strong and sweet smell recalling *Lepista irina* (Fr.) H.E. Bigelow (Kühner 1956, Klán 1983, Bon 1991, Boekhout 1988, 1999; Krieglsteiner 2001, Fontenla et al. 2003). Boekhout (1988, 1999) considered it a variety of *M. exscissa* and Krieglsteiner (2001) cited it as only an accidental *M. exscissa* phenotype; our molecular data (P%IV between *M. iris* and *M. exscissa* sequences = 98.7) clearly support Krieglsteiner. With an intraspecific variability lower than 3% (Nilsson et al. 2008), *M. iris* should be considered a form of *M. exscissa*. *Melanoleuca diverticulata* differs from *M. exscissa* mainly by the presence of elements with small outgrowths in the pileipellis (Moreno & Bon 1980). The P%IV between *M. diverticulata* and *M. exscissa* ANC M0207 and ANC M0208 equals 97.9. Our data show that *M. substrictipes* var. *sarcophylla,* which Kühner (1978) distinguished from the typical variety based on its pinkish lamellae (Kühner 1978, Bon 1991), is more closely related to *M. exscissa* than to *M. substrictipes* Kühner (Fig. 2) and should be considered a form of *M. exscissa* with coloured lamellae (P%IV = 98.5).

Therefore, we propose the following new combinations:

Melanoleuca exscissa f. ***iris*** (Kühner) Fontenla, Para & Vizzini, **comb. nov., stat. nov.**

MycoBank MB 563141

≡ *Melanoleuca iris* Kühner, Bull. Soc. Linn. Lyon 25(7): 181 (1956).

The holotype of *M. iris* apparently missing from G (herbarium where Kühner's collections of *Melanoleuca* are kept; Fontenla & Para, unpublished data).

Melanoleuca exscissa f. ***sarcophylla*** (Kühner) Fontenla, Para & Vizzini, **comb. nov., stat. nov.**

MycoBank MB 563142

≡ *Melanoleuca substrictipes* var. *sarcophylla* Kühner, Bull. Soc. Linn. Lyon 47(1): 52 (1978).

Holotype (G - K 65-24), with morphological features coincident with those of our sequenced collection (ANC M0209; Fontenla & Para, unpublished data).

Melanoleuca exscissa f. ***diverticulata*** (G. Moreno & Bon) Fontenla, Para & Vizzini, **comb. nov., stat. nov.**

MycoBank MB 563143

≡ *Melanoleuca diverticulata* G. Moreno & Bon, Docum. Mycol. 11(41): 35 (1980).

Holotype (MA - 3662), with morphological features coincident with those of our sequenced collection (ANC M0206; Fontenla & Para 2007).

Subclade A2.2 (0.95 BPP, 77% MLB) The two *M. substrictipes* collections are very similar (P%IV = 99.1). The first (ANC M0214) has only urticoid *exscissa*-type cheilocystidia, whereas the second (MCVE 13934) possesses

both *brevipes*- and *exscissa*-type cheilocystidia. Following Bon (1991) the two molecularly clearly conspecific collections would be referred to two different subsections: the first in the subsect. *Exscissae* (as *M. pseudoevenosa* Bon ex Bon & G. Moreno) and the second in subsect. *Grammopodiae*. The holotype (G-K 66-13) is characterized by both types of urticiform cheilocystidia (Fontenla & Para 2011).

Subclade A2.3 (1.0 BPP, 100 % MLB) The two *M. verrucipes* (Fr.) Singer sequences (GenBank DQ490642 and MCVE 09962) are almost identical (P%IV = 99.8). The black dotted stipe readily diagnoses the species (Bon 1991, Boekhout 1999, Gasparini 2001, Fontenla et al. 2003).

Clade A3 (0.53 BPP, / MLB), cheilocystidia absent or urticocystidia mainly of the *brevipes*-type. Both pileus and stipe usually (except for *M. robertiana*) dark coloured and lamellae white to whitish. This clade encompasses the two acystidiate species, *M. robertiana* Bon and *M. stridula* (Fr.) Singer, and subclades A3.1 and A3.2.

Subclade A3.1 (0.66 BPP, / MLB) comprises four acystidiate taxa, *M. striimarginata* Métrod ex Bon, *M. "paratristis," M. graminicola* (Velen.) Kühner & Maire, and *M. angelesiana* A.H. Sm. The first three taxa (subg. *Acystis* sect. *Acystis* in Bon 1991) are morphologically and molecularly (P%IV = 98.2) closely related: they share a collybioid habit, white lamellae, and a whitish stipe, differing mainly in pileus colour and could probably be reduced to only one species, *M. graminicola* (which would have nomenclatural priority). *Melanoleuca angelesiana* (the 2 sequences with a P%IV = 99.9) is an independent species (P%IV = 94.3), distinguished by a tricholomatoid habit, grey lamellae, and greenish-brown stipe. The two Italian collections (ANC M0203, ANC M0204) show features consistent with the protologue (Smith 1944) and holotype (MICH 14633) of this North American species (Fontenla & Para, unpub. data).

Subclade A3.2 (0.99 BPP, 89 % MLB) encompasses *M. decembris* Métrod ex Bon and *M. pseudopaedida* Bon. Bon (1991) classified these species as acystidiate (subg. *Acystis*, sect. *Decembres*), but Fontenla & Para (2008) found numerous urticiform cheilocystidia when they studied the type collections. Both taxa are characterized by a dark (dark brown-grey to blackish) pileus and grey lamellae (Bon 1991). Molecular analysis supports *M. pseudopaedida* as conspecific with *M. decembris* (P%IV = 98.1).

Clade A4 (0.54 BPP, / MLB), cheilocystidia absent or both *brevipes*- and *exscissa*-type urticocystidia, comprising the acystidiate *M. microcephala* (P. Karst.) Singer and A4.1 and A4.2 subclades. Bon (1991) described *M. microcephala* as having urticiform cystidia, but Fontenla and Para (unpub. data) found Karsten's type collection (H 1604) to be acystidiate.

Subclade A4.1 (1.0 BPP, 99 % MLB) contains *M. pseudoluscina*, *M.* sp. 12, and *M.* sp. 18. The seven sequences show a P%IV of 97.1. The P%IV (98.6) between *M.* sp. 18 and *M. pseudoluscina* sequences indicates that *M.* sp. 18 is an acystidiate form of *M. pseudoluscina*. Our analysis support *M. pseudoluscina* as an independent species and not a variety of *M. rasilis* (subclade A1.2) as stated by Boekhout (1988, 1999).

Subclade A4.2 (1.0 BPP, 99 % MLB) encompasses *M. paedida*, *M.* sp. 2, and *M. electropoda*. The two *M. paedida* collections are consistent with the protologue and the observations by Fontenla et al. (2003). *Melanoleuca* sp. 2 is an acystidiate form of *M. paedida*. *Melanoleuca electropoda* was reported by Bon (1991) as a macrocystidiate species (subg. *Melanoleuca*, sect. *Oreineae*); after finding typical urticoid cheilocystidia in the type collection, Fontenla et al. (2009) considered *M. rufipes* Bon a later synonym. As *M. electropoda* differs from *M. paedida* only in an orange red (instead of brown) stipe base and context, we reduce it to a form of *M. paedida* based on molecular data (P%IV = 98.9).

Melanoleuca paedida* f. *electropoda (Maire & Malençon) Fontenla, Para & Vizzini, **comb. nov., stat. nov.**

MycoBank MB 563144

≡ *Melanoleuca electropoda* Maire & Malençon, Fl. Champ. sup. Maroc 2: 77 (1975).

Clade A5 (1.0 BPP, 99% MLB) (= sect. *Cognatae*), macrocystidiate taxa with bright coloured pilei.

Melanoleuca cognata (Fr.) Konrad & Maubl. and *M. arcuata* (Bull.) Singer are two independent species (P%IV = 95.4). *Melanoleuca arcuata* differs by having a brown pileus and yellow-ochre lamellae only at maturity. The collection named *M.* sp. 10 is probably referable to a new species (P%IV = 93.1), but its macromorphological data are lacking.

Subgenus *Melanoleuca* (Clade B): clades B1-B5 + *M. friesii* (Fr.) Singer (a species characterized by a dark-brown blackish, slate-grey pileus, whitish lamellae and mainly lageniform cystidia).

Clade B1 (1.0 BPP, 95% MLB), macrocystidia both fusiform and lageniform, pileus mainly grey-brown and lamellae white to grey. It encompasses the subclades B1.1, B1.2, B1.3 + 4 *M.* sp. (*M.* sp. 3, *M.* sp. 4, *M.* sp. 5 and *M.* sp. 11).

Subclade B1.1 (0.79 BPP, 75 % MLB) comprises *M. subpulverulenta* (Pers.) Singer, *M. melaleuca* and *M. nivea* 1. The four collections are closely related (P%IV = 98.6) despite their morphological differences. *Melanoleuca subpulverulenta* is characterized by a pruinose matte grey pileus, pruinose stipe,

and dark context; *M. melaleuca* has a grey-brown non-pruinose pileus, non-pruinose stipe, and whitish context sometimes darkening towards the stipe base. The *M. nivea* 1 collection (ANC M0177) we originally referred to *M. nivea* due to its whitish colours (sect. *Alboflavidae*) and <5 cm pileus represents only an albinic form of the *M. subpulverulenta*/*M. melaleuca* complex; its features nonetheless match those of the *M. nivea* holotype (PC–2434) (Fontenla & Para, unpublished data).

Subclade B1.2 (0.56 BPP, 45 % MLB) comprises *M.* sp. 6, *M. nivea* 3, and *M. robusta*, with the latter distinguished by a grey-brown pileus, grey lamellae, brown context, caespitose growth, and mainly fusiform macrocystidia. *Melanoleuca nivea* 3 is very close to an unidentified collection (*M.* sp. 6) characterized by a grey pileus.

Subclade B1.3 (0.62 BPP, 73 % MLB) consists of two molecularly closely related taxa (P%IV = 98.8), *M. "lanipes"* and *M. heterocystidiosa* (Beller & Bon) Bon that have both lageniform and fusiform macrocystidia. *Melanoleuca lanipes,* which has dark grey-brown lamellae, strongly longitudinally striate dark grey stipe covered with woolly flocci, and blackish stipe base, resembles *M. cognata* but with a tomentose-wooly stipe.

Clade B2 (0.52 BPP, / MLB), mainly lageniform cystidia, longitudinally striate and brown to dark brown stipe.

Clade B2 is formed by *M. albifolia*, *M. atripes*, *M. nivea* 2, *M.* sp. 1 and *M.* sp. 8. All six sequences are closely related (P%IV = 97.6). Morphologically, *M. albifolia* is well characterized by a rather small size (2.5-5 cm diam), dark sepia-brown pileus, white lamellae, grey-brown stipe, and lageniform cystidia (Boekhout 1988, 1999; Bon 1991); *M. atripes* has a hygrophanous blackish brown pileus, dark brown stipe, yellowish beige lamellae, and mainly fusiform cystidia (Boekhout 1988, 1999; Bon 1991). In contrast, our *M. atripes* collection (ANC M0180) shows mainly lageniform cystidia.

The sequence analysis did not support any independent species in the clade. *M. nivea* 2 is probably an albinic form of the *M. albifolia*/*M. atripes* complex while *M.* sp. 1 is the only acystidiate collection nested within subgen. *Melanoleuca* (clade B).

Clade B3 (1.0 BPP, 92% MLB), macrocystidia mainly fusiform, basidiome white to cream-whitish (sect. *Alboflavidae*).

Clade B3 comprises *M. strictipes* (P. Karst.) Jul. Schäff., *M. evenosa* (Sacc.) Konrad, and *M. subalpina* (Britzelm.) Bresinsky & Stangl. The five samples are very closely related (P%IV = 98.4) and have characters consistent with those of the original collections. Bon (1991) circumscribed the species in sect. *Alboflavidae* mainly based on Qm value, odour, pileus size, and stipe ornamentation. We

have now examined morphologically the holotype of *M. strictipes* (H 2432, Fontenla & Para, unpubl.), lectotype of *M. subalpina* (Fontenla & Para 2008), and eight original *Tricholoma cnista* sensu Bresadola (= *M. evenosa*) collections (TR, BPI). This, combined with our thorough study of the original diagnoses and phylogenetic analysis, lead us to conclude that *M. strictipes*, *M. evenosa,* and *M. subalpina* are conspecific, with the name *M. strictipes* having priority.

Clade B4 (0.52 BPP, / MLB), cystidia fusiform or lageniform, pileus small (up to 5 cm broad) and not dark-coloured, lamellae whitish-cream, context white.

Clade B4 comprises *M. oreina* (Fr.) Kühner & Maire, *M. bataillei* Malençon, *M.* sp. 7, and *M.* sp. 9. The first two species, which are phenetically very similar, differ mainly on cystidial shape. Bon (1991) placed *M. oreina* (with fusiform cystidia) into sect. *Oreinae* and *M. bataillei* (with mainly lageniform cystidia) into sect. *Melanoleuca*. Molecularly, they are quite distinct (P%IV = 92.5).

Clade B5 (0.84 BPP, 54% MLB) consists of the three *M. cinereifolia* (Bon) Bon sequences (P%IV = 100).

Melanoleuca cinereifolia is distinguished by a habit reminiscent of *Clitocybe nebularis* (Batsch) P. Kumm., always grey lamellae, short stipe, strictly lageniform (M2) cystidia, and growth in sand dunes.

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Geastrum species from the Amazon Forest, Brazil

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Abstract—Six *Geastrum* species are reported from the Amazon forest: *G. entomophilum*, *G. fimbriatum*, *G. javanicum*, *G. lageniforme*, *G. lilloi*, and *G. saccatum*. *Geastrum javanicum* and *G. lilloi* represent first records from Brazil. Descriptions and illustrations of the species and SEM images of microstructures are given.

Key words— *Basidiomycota*, *Geastraceae*, gasteromycetes, taxonomy

Introduction

The family *Geastraceae* was first described by Corda (1842) as the *Geastrideae* and subsequently classified in the *Lycoperdales* (Lloyd 1902, Ponce de León 1968, Krüger & Chagas 2008), the *Phallales* (Hibbett et al. 1997) and, based on molecular data, recently in the *Geastrales* (Hosaka et al. 2006). With 43 species reported, the *Geastraceae* is the second-most represented gasteromycete family in Brazil (Trierveiler-Pereira & Baseia 2009). The *Geastraceae* comprise eight genera: *Geastrum*, *Myriostoma*, *Trichaster*, *Geasteropsis*, *Phialastrum*, *Pyrenogaster*, *Radiigera,* and *Terrostella*, with *Geastrum* being the largest (Sunhede 1989) with 50 species recognized worldwide (Kirk et al. 2008).

Tropical regions contain most of the fungi still unknown to science. Tropical Amazonian rainforests account for 25% of the remaining forests worldwide and cover nearly half of Brazil, representing enormous strategic value (Braga-Neto et al. 2008). The Amazon biome, which covers 4,871,000 km^2 (INPE

2004), extends from the Atlantic Ocean to the eastern slopes of the Andes up to approximately 600 m altitude, encompassing parts of nine South American countries and with 69% falling within the boundaries of Brazil (Ab'Sáber 1977).

The mycobiota of the Brazilian Amazon has been poorly studied and needs to be more thoroughly investigated. However, the following existing studies are noteworthy: Singer & Araujo (1979), Aguiar (1984), Singer (1984), Singer & Aguiar (1986), Capelari & Maziero (1988), and Bononi (1992). Several studies on gasteromycetes in the Amazon region have been conducted: Berkeley & Cooke (1876), Hennings (1904), Capelari & Maziero (1988), Trierveiler-Pereira et al. (2009). The purpose of this paper is to contribute to the existing knowledge of this mycobiota, with special reference to the family *Geastraceae*.

Material & methods

Material was collected from 1995 to 2008 at the Reserva Nacional de Caxiuanã, in the municipality of Melgaço, Pará state, Amazonas, Brazil, and deposited in the herbaria of the Museu Paraense Emílio Goeldi (MG), Belém, Pará, and UFRN, Universidade Federal do Rio Grande do Norte, Natal. These collections are considered reference depositories for the study of Amazonian biodiversity (Braga-Neto et al. 2008). Additional Brazilian specimens held in UFRN were also studied. Macro- and microscopic characters were studied according to Miller & Miller (1988), Sunhede (1989), Ponce de León (1968), Soto & Wright (2000), Baseia & Milanez (2002), Calonge et al. (2000), Calonge & Mata (2004), Baseia & Calonge (2006), Leite & Baseia (2007), and Leite et al. (2007). Color citations were based on Kornerup & Wanscher (1978). Microstructures were analyzed in sections mounted on slides, using 5% KOH, and SEM was used to study basidiospore ornamentation.

Results

Geastrum entomophilum Fazolino, Calonge & Baseia, Mycotaxon 104: 450 (2008).

Fig. 1

Expanded basidiome epigeous, 7.3 cm broad. Exoperidium semi-hygroscopic, arched, split into 6 rays, recurved under the exoperidial disc; mycelial layer light orange (6A5), covered with adhering humus, leaf and sand; fibrous layer light orange (6A4); pseudoparenchymatous layer brown (6F4). Endoperidium sessile, globose to subglobose, 2 cm diam., ornamentation formed by fascicles of worm-shaped surface hyphae (Fig. 1b), greyish brown (5D3). Peristome fibrillose, concolorous with rest of endoperidium, indistinctly delimited. Gleba brownish grey (5F2). Basidiospores globose, ornamentation more or less columnar, 3.5–4 µm diam. Capillitial hyphae with verrucose surface, 3–3.1 µm diam.

Substrate & distribution— Sandy soil. Known only from South America, this is the first record of this species from the Amazon rainforest.

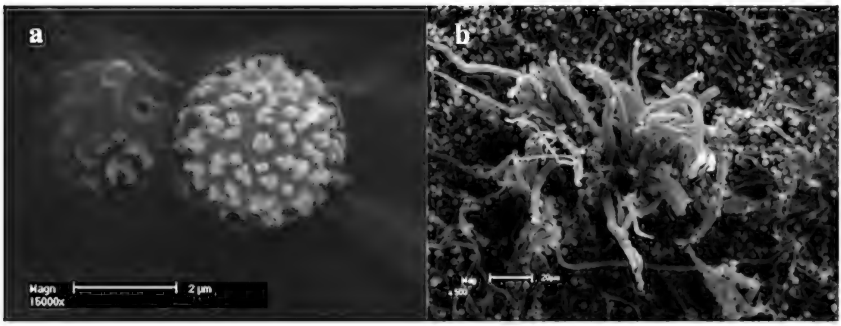

Fig. 1. *Geastrum entomophilum*. a. Basidiospores; b. Endoperidial hyphal fascicle.

Specimen examined: **BRAZIL. Pará**: Melgaço, Floresta Nacional de Caxiuanã, Estação Científica Ferreira Penna, 20.II.1997, col. H. Sotão & al. (MG 199685).

Additional specimen examined: **BRAZIL. Rio Grande do Norte**: Natal, Parque Estadual Dunas do Natal, 15.VII.2006, col. E.P. Fazolino & A.G. Leite. (UFRN-Fungos 354).

Taxonomic remarks—*Geastrum entomophilum* is recognized by its sessile endoperidium, verrucose surface with vermiform hyphae in fascicles, and fibrillose and lacerated peristome, non-delimited when older. In the original description (Fazolino et al. 2008) the small beetles (*Coleoptera*) observed inside the gleba led to the name '*entomophilum*.' Beetles were not observed by Trierveiler-Pereira & Baseia (2010).

Geastrum fimbriatum Fr., Syst. mycol. 3(1): 16 (1829). Fig. 2

Basidiomata epigeous when young, globose to depressed-globose, 1.7 cm diam. × 2 cm high, epigeous at maturity, 2–2.5 cm broad, 0.4–0.7 cm high. Exoperidium non-hygroscopic, saccate, splitting into 4–6 rays; mycelial layer dark blond (5D4); fibrous layer adherent, greyish yellow (4B3); pseudoparenchymatous layer brown (5F5). Endoperidium sessile, globose to subglobose, 0.8–2 cm diam., brown (6E4). Peristome absent to fibrillose. Gleba dark brown (6F5); columella present. Basidiospores 3–3.5 µm diam., globose, ornamentation columnar. Capillitial hyphae thick-walled, with surface debris and verrucose, 3.5–5 µm diam.

Substrate & distribution— Sandy soil. Known from South America, Central America, North America, Europe, and Australasia.

Specimen examined: **BRAZIL. Pará**: Melgaço, Floresta Nacional de Caxiuanã, Estação Científica Ferreira Penna, 30.V.1997, col. H. Sotão & al. (MG-199687, UFRN-fungos 1465).

Additional specimen examined: **BRAZIL. Rio Grande do Norte**: Parque Estadual Dunas do Natal, 02.VI.2007, col. E.P. Fazolino & B.D.B. Silva (UFRN-Fungos 337).

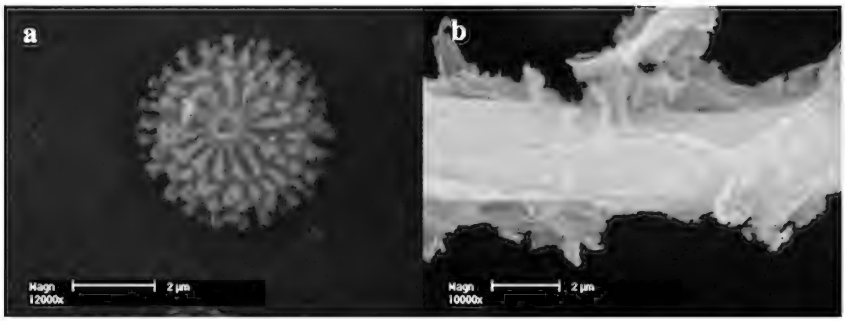

FIG. 2. *Geastrum fimbriatum*. a. Basidiospore; b. Capillitial hypha.

TAXONOMIC REMARKS—*Geastrum fimbriatum* strongly resembles *G. saccatum*, mainly with respect to its basidiospore ornamentation, but it also differs in having smaller spores, with those of *G. saccatum* 4–6 µm diam. (Soto & Wright 2000). A marked characteristic in this species observed by both Sunhede (1989) and us is the interspersed hyphal formation in the mycelial layer.

This is the second record of *C. fimbriatum* from the Amazon rainforest; the first was made by Trierveiler-Pereira et al. (2009).

Geastrum javanicum Lév., Annls Sci. Nat., Bot., sér. 3, 5: 161 (1846). FIG. 3-4

Basidiomata epigeous when young, 0.7–1.6 cm broad, 0.5–1.5 cm high, caespitose on a subiculum, white yellowish (4A1–4A2); expanded basidiome epigeous. Exoperidium hygroscopic, 1.5–1.9 cm diam. when open, split into 5–8 recurving rays; mycelial layer velvety, separates at maturity, greyish brown (5D3); fibrous layer light yellowish (5C4); pseudoparenchymatous layer brownish orange (5C4). Endoperidium brown (6F4) to brownish grey (7E2), sessile, globose, 1–1.2 cm diam. Peristome delimited, fibrillose, concolorous with rest of endoperidium. Gleba greyish brown (6F3); columella present.

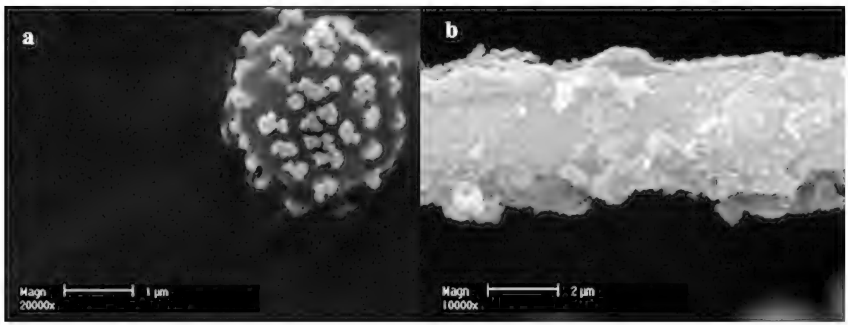

FIG. 3. *Geastrum javanicum*. a. Basidiospore; b. Capillitial hypha.

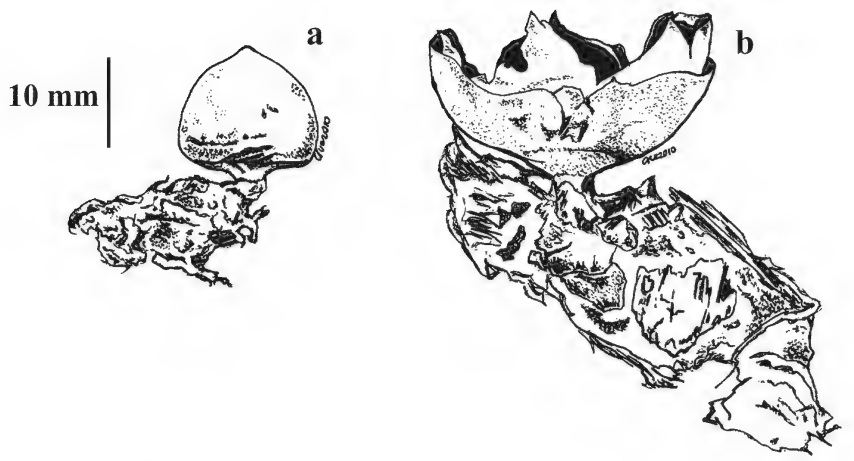

Fig. 4. *Geastrum javanicum.* a. Immature basidioma; b. Mature basidioma.

Basidiospores globose, 2.5–3 µm diam., with irregular warts. Capillitial hyphae, 3–4 µm diam., surface rugose, septate and branched.

Substrate & distribution—Decaying wood. Known from South America, Central America, North America, Africa, Australasia, Asia.

Specimen examined: **BRAZIL. Pará**: Melgaço, Floresta Nacional de Caxiuanã, Estação Científica Ferreira Penna, 12.IV.1995, col. H. Sotão & al. (MG nº 0149528).

Additional specimen examined: **BRAZIL. Rio Grande do Norte**: Parnamirim, Mata do Jiqui, 22.VI.2007, col. E.P. Fazolino & al. (UFRN-Fungos 550).

Taxonomic remarks—*Geastrum javanicum* is generally found growing in clusters, forming whitish subiculum on dead wood, leaf litter, and occasionally in sand impregnated with decomposing organic matter (Ponce de León 1968). A diagnostic character is the velvety exoperidium that detaches itself from the mycelial layer when the basidioma is mature. Ponce de León (1968) reviewed the holotypes of *G. velutinum* Morgan and *G. javanicum* and considered them to be synonyms. However, on the basis of priority, *G. javanicum* is the correct name to use. Sunhede (1989) did not study the type of material of *G. javanicum*, and therefore did not confirm its synonymy with *G. velutinum*.

This species has been reported from Brazil by Sobestiansky (2005), de Meijer (2006), and Sydow & Sydow (1907) as *G. velutinum*, but this is the first record from the Amazon rainforest.

Geastrum lageniforme Vittad., Monograph. Lyc.: 16 (1842). Fig. 5

Mature basidiomata 1–2.4 cm broad, 0.8–1.4 cm high. Exoperidium non-hygroscopic, saccate with 4–6, long, slender-tipped rays, extended or recurved

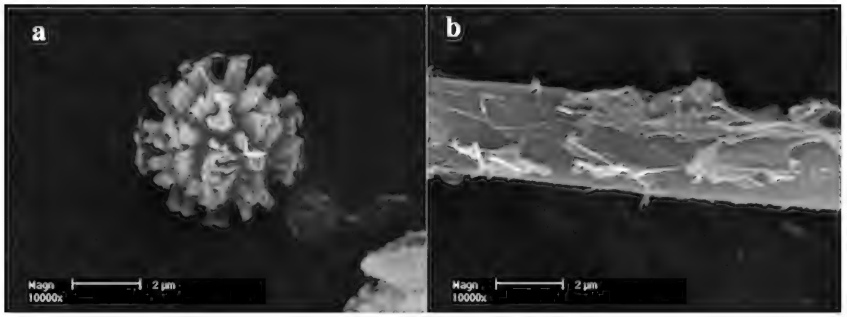

FIG. 5. *Geastrum lageniforme*. a. Basidiospore; b. Capillitial hypha.

under the exoperidial disc and the rays are markedly tapered and pointed; mycelial layer blond (4C4), with longitudinal fissures; fibrous layer pale orange (5A3); pseudoparenchymatous layer yellowish brown (5F8). Endoperidium dark blond (5D4), sessile, globose to subglobose, 0.8–1.2 cm diam. Peristome fibrillose, delimited, conical to mammiform. Gleba pale orange (5A3); columella present. Basidiospores globose 4.5–5 µm diam., ornamentation columnar. Capillitial hyphae thick-walled, 2–2.8 µm diam.

SUBSTRATE & DISTRIBUTION—Sandy soil. Known from South America, Central America, North America, and Europe; this is the first record of this species from the Amazon rainforest.

SPECIMEN EXAMINED: **BRAZIL. PARÁ**: Melgaço, Floresta Nacional de Caxiuanã, Estação Científica Ferreira Penna, 19.II.1997, col. H. Sotão & al. (MG 199683).

ADDITIONAL SPECIMEN EXAMINED: **BRAZIL. RIO GRANDE DO NORTE**: Natal, Parque Estadual Dunas do Natal, 29.VII.2006, col. E.P. Fazolino & al. (UFRN-fungos 332).

TAXONOMIC REMARKS—This species is characterized by a saccate exoperidium, sessile endoperidium and fibrillose, well-delimited peristome. It is very similar to and often confused with *G. saccatum* and *G. triplex* Jungh. (Soto & Wright 2000). Fries (1829) mentions nothing about grooves in the mycelial layer in *G. saccatum*. In *G. lageniforme*, after dehiscence of the exoperidium, the rays are markedly longitudinally cracked. According to Sunhede (1989), *G. triplex* exhibits an endoperidial body of 7.5–19.5 mm diam., while in *G. lageniforme* it reaches 11–54 mm.

Geastrum lilloi L.S. Domínguez, Mycologia 88: 858 (1996). FIGS. 6-7

Young basidiomata epigeous, 0.8 cm broad, 0.4 cm high, surface irregular, yellowish white pale (2A2), caespitose, growing on a pale yellow (4A3) subiculum; mature basidiomata 1.1–1.4 cm diam. Open exoperidium saccate, splitting into 5–6 rays, hygroscopic; mycelial layer with irregular surface, pale yellowish white (2A2); fibrous layer white (6D4); pseudoparenchymatous layer

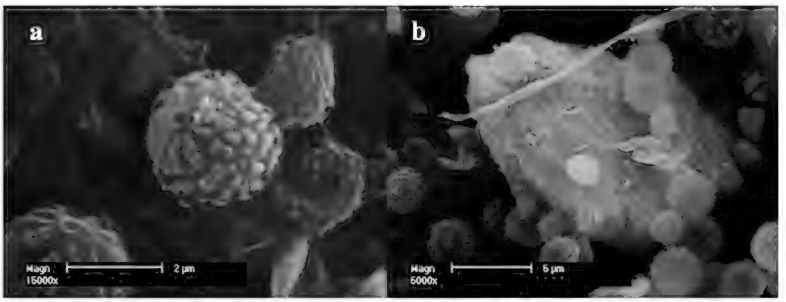

FIG. 6. *Geastrum lilloi*. a. Basidiospores; b. Mycosclereid.

brown (5F7). Endoperidium brown (6E4), sessile, globose, 0.6–0.8 cm diam. Peristome fibrillose, mammiform, greyish brown (5D3), indistinctly delimited. Gleba dark brown (6F3); mycosclereids present, up to 10.2 × 15.9 µm diam.; columella present. Basidiospores globose, 2.8–3.5 µm diam., ornamentation irregularly columnar. Capillitial hyphae 1.8–2 µm. diam., covered with encrusting surface debris.

SUBSTRATE & DISTRIBUTION—Woody debris. Known from South America (Argentina, Brazil).

SPECIMEN EXAMINED: **BRAZIL. Pará**: Melgaço, Floresta Nacional de Caxiuanã, Estação Científica Ferreira Penna, 29.V.1997, col. H. Sotão & al. (MG 199686).

TAXONOMIC REMARKS—Distinguishing characteristics of *G. lilloi* are its small size, gregarious and xylophilous habitat, velvety mycelial layer, the presence of mycosclereids in the gleba, the endoperidium with a fibrillose, mammiform pore, and epigeal development (Domínguez de Toledo 1996). *Geastrum schweinitzii* (Berk. & M. A. Curtis) Zeller can easily be confused with *G. lilloi* because it also is small-sized and grows on a subiculum on wood. However,

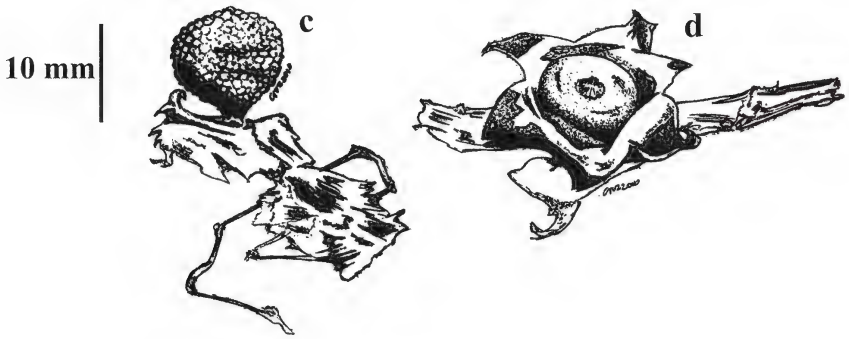

FIG. 7. *Geastrum lilloi*. a. Immature basidioma; b. Mature basidioma.

these two species differ in terms of the non-hygroscopic exoperidium, obovate fruit body, peristome without a ring, and smooth to velvety mycelial layer in *G. schweinitzii*.

This is the first record of *G. lilloi*, first described from Argentina, from Brazil.

Geastrum saccatum Fr., Syst. Mycol. 3(1): 16 (1829). FIG. 8

Mature basidiomata 0.5–2 cm diam. Exoperidium non-hygroscopic, saccate, splitting into 5–7 rays; mycelial layer encrusted with debris of soil and leaf litter, light orange (6A4); pseudoparenchymatous layer brown (6E4); fibrous layer adhered to pseudoparenchymatous layer, pale orange (6A3). Endoperidium brownish beige (6E3), sessile, globose, 0.8–1.2 cm diam. Peristome well delimited, fibrillose, conical to mammiform, concolorous with rest of endoperidium. Gleba greyish brown (6F3); columella present. Basidiospores globose to subglobose, 3.6–4.5 µm diam., ornamentation rather irregular columnar. Capillitial hyphae 3–3.5 µm diam, covered with small wart-like surface outgrowths.

SUBSTRATE & DISTRIBUTION— Sandy soil. Known from South America, Central America, North America, Africa, Asia, Australasia, and Europe.

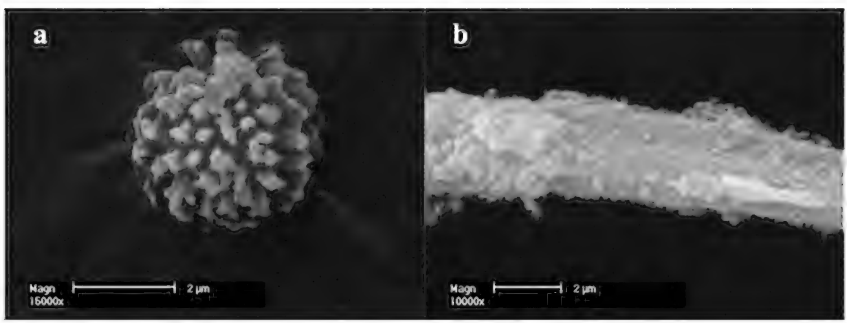

FIG. 8. *Geastrum saccatum*. a. Basidiospore; b. Capillitial hypha.

SPECIMEN EXAMINED: **BRAZIL. PARÁ**: Melgaço, Floresta Nacional de Caxiuanã, Estação Científica Ferreira Penna, 19.II.1997, col. H. Sotão & al. (MG-199684).

ADDITIONAL SPECIMEN EXAMINED: **BRAZIL. RIO GRANDE DO NORTE**: Natal, Parque Estadual Dunas do Natal, 15.VII.2006, col. E.P. Fazolino & al. (UFRN-Fungos 334).

TAXONOMIC REMARKS—*Geastrum saccatum* is characterized mainly by its well-delimited, fibrillose peristome and sac-like, sessile endoperidium. It resembles *G. lageniforme* but differs in its smaller and wider rays and a mycelial layer lacking longitudinal striations (Soto & Wright 2000). Macroscopically it resembles young *G. triplex* specimens, but at maturity the pseudoparenchymatous layer of

G. triplex detaches from the fibrous layer. Another marked difference between these two species is the much smaller basidioma of *G. saccatum*.

This is the second record of *G. saccatum*, first recorded by Hennings (1904), from the Amazon rainforest.

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The authors thank the reviewers, Johan C. Coetzee and Admir Giachini, for their valuable suggestions. We express our gratitude to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for partial financial support; to CTPETRO-INFR, FINEP/LIEM for collaboration with the SEM and MPEG and to Estação Científica Ferreira Penna for fieldwork logistical support.

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Hypoderma siculum sp. nov. from Italy

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Abstract — *Hypoderma siculum* is described and illustrated as a new species from southeast Sicily (Italy) that occurs on remnants of *Ferula communis* (*Apiaceae*). Its ecology and taxonomic and phylogenetic relationships are discussed.

Key words — morphology, taxonomy, ITS phylogeny

Introduction

Field investigation of the *Ascomycetes* of Sicily occurring on decaying remnants of *Ferula communis* (Lantieri 2009) revealed a new *Hypoderma* species, described here as *Hypoderma siculum*.

Materials & methods

Collections of the new species were made between 2007 and 2010 at an elevation of 700 m a.s.l. in southeast Sicily. Morphological and microscopic examinations were carried out on fresh material and on dried specimens rehydrated in water. Observations and measurements were made in water and Melzer's reagent. Ascus and ascospore size ranges from the holotype were based on 50 measurements, using an Optika optical microscope (model BK 1301), with 40× or 100× (oil immersion) objectives. All voucher specimens were deposited in the fungarium of the Royal Botanic Gardens, Kew K(M) and in the fungal reference collection of Landcare Research in New Zealand (PDD).

DNA was extracted separately from two sets of four fruiting bodies taken from each of two separate blackened areas on leaf pieces within the isotype specimen (PDD 99894), using REDExtract-N-Amp Plant PCR Kits (Sigma, USA). The perithecia were

Table 1. Specimens used in phylogenetic analysis

Species	Country of origin, host	Collection voucher	Genbank acc. no.
Coccomyces australis	Argentina, *Desfontainia spinosa*	ICMP 16772	EF191241
Hypoderma commune	Sweden, ?*Euphorbia* sp.	Hanson 2006-451 (UPS)	JF690769 *
H. cordylines	New Zealand, *Cordyline australis*	ICMP 17344 (ex-holotype culture)	JF683421 *
	New Zealand, *Phormium* sp.	ICMP 17359	JF683420 *
H. hederae	Scotland, *Hedera helix*	Lantz & Minter 421 (UPS)	JF690770 *
H. rubi	China, host unknown	unknown	GU138735
	China, host unknown	unknown	GU138736
	China, host unknown	unknown	GU138738
	China, host unknown	unknown	GU138739
	China, host unknown	unknown	GU138741
	China, host unknown	unknown	GU138743
	China, host unknown	unknown	GU138745
	China, host unknown	unknown	GU138746
	China, host unknown	unknown	GU138750
	China, host unknown	unknown	GU138751
	China, host unknown	unknown	GU367895
	China, host unknown	unknown	GU367898
	New Zealand, *Coprosma* sp.	ICMP 18768	JF683417 *
	New Zealand, *Melicytus ramiflorus*	ICMP 18325	JF 683418 *
	New Zealand, *Pseudopanax* sp.	ICMP 17349	JF683416 *
	New Zealand, *Rubus cissoides*	ICMP 17339	JF 683419 *
	Sweden, *Rubus lindebergii*	Hanson 2002-230 (UPS)	JF690771 *
H. siculum	Italy, *Ferula communis*	PDD 99894 (isotype)	JF683424 *
H. stephanandrae	China, *Stephanandra chinensis*	unknown	GU138753
H. vincetoxici	Sweden, *Vincetoxicum hirundinaria*	Lantz 405 (UPS)	JF690772 *
Lophodermium agathidis	New Zealand, *Agathis australis*	ICMP 18327	JF683423 *
	New Zealand, *Metrosideros fulgens*	ICMP 17345	JF683422 *
L. eucalypti	New Zealand, *Leptospermum scoparium*	ICMP 16796	EF191235
L. gamundiae	Argentina, *Nothofagus dombeyi*	ICMP 16797	EF191239

* = newly generated for this study).
UPS = Uppsala University; ICMP = International Collection of Microorganisms from Plants (maintained by Landcare Research)

ground in extraction buffer with a plastic pestle in the Eppendorf tube. Following this, DNA extraction and PCR were carried out following manufacturer's instructions. ITS sequences were obtained from each extract following the methods of Johnston & Park (2005).

Using ClustalW (Thompson et al. 1994), our newly generated sequences were aligned with sequences deposited in Genbank as *Hypoderma rubi* or those that closely matched *H. rubi*. Because of the paucity of available data, ITS sequences were generated from several additional *Hypoderma* spp. from New Zealand, with DNA extracted from cultures grown from germinating ascospores. Other taxa included in the analysis were members *Lophodermium eucalypti/Coccomyces tumidus* clade recognized by Lantz et al. (2011) that formed a sister relationship to the core *Hypoderma* clade, and *Lophodermium agathidis* as the outgroup.

The taxa included are listed in Table 1, along with the Genbank accession numbers of our newly generated sequences. A Bayesian phylogenetic analysis was performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) with gaps treated as missing data, using the K80+I+G model which was selected using the AIC method in MrModelTest 2.3 (Nylander 2004). The data set was run with 2 chains for 10 million generations, trees sampled every 1000 generations with a burn-in of 25%. Bayesian posterior probabilities were obtained from 50% majority rule consensus trees.

Taxonomy

Hypoderma siculum Lantieri, P.R. Johnst. & Medardi **sp. nov.** Plates 1–2

MycoBank MB 563145

Ascomata erumpentia inter amplas variiformes nigras areas, haud compositas, supra plantae superficiem, haud consociata cum zonae lineis. Partes nigrae ex 1–2 stratis hypharum parietibus obscuris et inflatis, formantibus amplum stratum, 5–8 µm latum sub plantae cuticula, constitutae. Aspectus in superficie: ascomata 1–1.5 × 0.5–0.8 mm, ellipsoidea vel leviter fusiformia vel navicularia si secta, extremitatibus repente rotundatis vel acutis, paene mersa in contextu plantae. Ascomata clausa parietibus nigrescentibus, sed plus minusve obscure griseis ad margines prominentes fissurae. Ascomata aperta per fissuram longitudinalem, se extendentem in totam longitudinem. Labra dilute griseola adsunt. Hymenium griseum caesio colore suffusum. Labri cellulae hyalinae ex hyphis inconstanter cylindricis, inflatis in nonnullis partibus, pariete crassa, cellulis 1 (raro 2) –septatis, 20–25 × 2 µm, mersis in involucro gelatino, constitutae. In recta media parte stroma tegens crassum usque ad 50 µm prope ascomatum centrum, sed tenuior ad margines atque tenens stroma basis ex strato inferiore et obscure brunneo texturae epidermoideae constitutum. Externa pars parietum cellularum stromatis tegentis obtecta materia densa, nigra, plus minusve granosa, ex particulis sine structura cellularum manifesta, sed nonnullis cellulis obscure polygonalibus, efformata. Excipulum abest. Subhymenium 20–40 µm crassum, ex textura intricata, hyphis 3–4 µm latis, parietibus tenuibus et hyalinis constitutum. Stroma basis 5–15 µm crassum, cum 3–4 stratis hypharum hyalinarum, 3–4 µm latarum, parietibus obscure brunneis et leviter inflatis, formantibus texturam intricatam. Ascosporae fusiformes vel cylindrico-fusiformes, nonnullae leviter curvae, 27–30 × 3–3.5 µm, laeves, hyalinae, haud septatae, guttulis haud compositis praeditae, saepe absentibus prope centrum, cinctae involucro gelatino, laxo, circiter 3 µm crasso. Asci clavato-stipitati, (85–)90–100 × 9–10 µm, apice rotundato, haud amyloidei, 8–sporigeri; sporae dispositae in parte superiore. Paraphyses numerosae, filiformes, 1–2 µm latae, emergentes usque ad 30 µm supra ascos, curvae, crispatae vel valde plicatae ad apicem, nonnullis parvis guttulis sparsis per totam longitudinem, sine septis. Specimina cum conidiis haud notata. Habitat: supra reliqua putrescentia Ferulae communis*; inventum solum hieme.*

Type: Italy. Sicily, vic. Vittoria (Ragusa), Riserva Naturale Orientata "Pino d'Aleppo", on decaying remnants of *Ferula communis*, 30/12/2007, Leg. Angela Lantieri (**holotype**, K(M) 167515; **isotype**, PDD 99894, GenBank JF683424).

Conidiomata not observed. Ascomata develop among large, irregularly shaped blackened areas on the plant surface, not associated with zone lines. The blackened areas have 1–2 layers of hyphae with walls dark and thick forming a 5–8 µm wide layer beneath the plant cuticle. In surface view ascomata 1–1.5 × 0.5–0.8 mm, elliptical to slightly fusiform or navicular in outline, with sharply rounded to acute ends, semi-immersed in the plant tissues. Closed ascomata with blackish walls, but more or less dark greyish near the prominent edges of the slit. Ascomata opening by a longitudinal slit, which extends along the whole length. Lips present, pale greyish. Hymenium grey with bluish reflexes. Lip cells hyaline, composed of irregularly cylindrical, in some points enlarged, thick-walled, 1 (rarely 2) –septate, cells, 20–25 × 2 µm embedded in a gelatinous sheath. In median vertical, covering stroma up to 50 µm thick near the centre of the ascomata, becoming thinner towards the edges, extending to the basal stroma, consisting of a inner layer of dark brown textura epidermoidea. Outer part of the walls of the cells in the covering stroma with dense, more or less granulose black matter, composed of small particles with no visible cellular structure, but some of them obscurely polygonal. Excipulum absent. Subhymenium 20–40 µm thick, composed of textura intricata, hyphae 1.5–2 µm diam. with hyaline, thin walls. Basal stroma 5–15 µm thick, comprises 3–4 layers of hyphae 3–4 µm diam. with walls dark brown and slightly thickened, forming a textura intricata. Ascospores fusiform or cylindrical-fusiform, some slightly curved, 27–30 × 3–3.5 µm, smooth, hyaline, aseptate, containing irregular oil drops often lacking near the middle, surrounded by loose gelatinous sheath about 3 µm thick. Asci clavate-stipitate, (85–)90–100 × 9–10 µm, apex rounded, not bluing in iodine, 8-spored, spores confined to the upper part. Paraphyses abundant, filiform, 1–2 µm diam., protruding up to 30 µm beyond the asci, curved, curled or remarkably bent at the apex, with some small oil-drops scattered along the length, without septa.

Habitat: On decaying remnants of *Ferula communis*, found only in winter.

Additional specimens examined: **ITALY. Sicily**, vic. Vittoria (Ragusa), Riserva Naturale Orientata "Pino d'Aleppo", 13/03/2008, [Leg. A. Lantieri] (K(M) 167516); 06/02/2009, [Leg. A. Lantieri] (K(M) 167517); 13/02/2010, [Leg. A. Lantieri] (K(M) 167518).

Discussion

Hypoderma siculum falls within the *Hypoderma* sensu stricto clade of Lantz et al. (2011). Genetic relations within this clade are poorly resolved in the ITS gene tree (Fig. 3). Despite this, *H. siculum* is genetically distinct from other named isolates within the clade, and this genetic isolation and its distinct

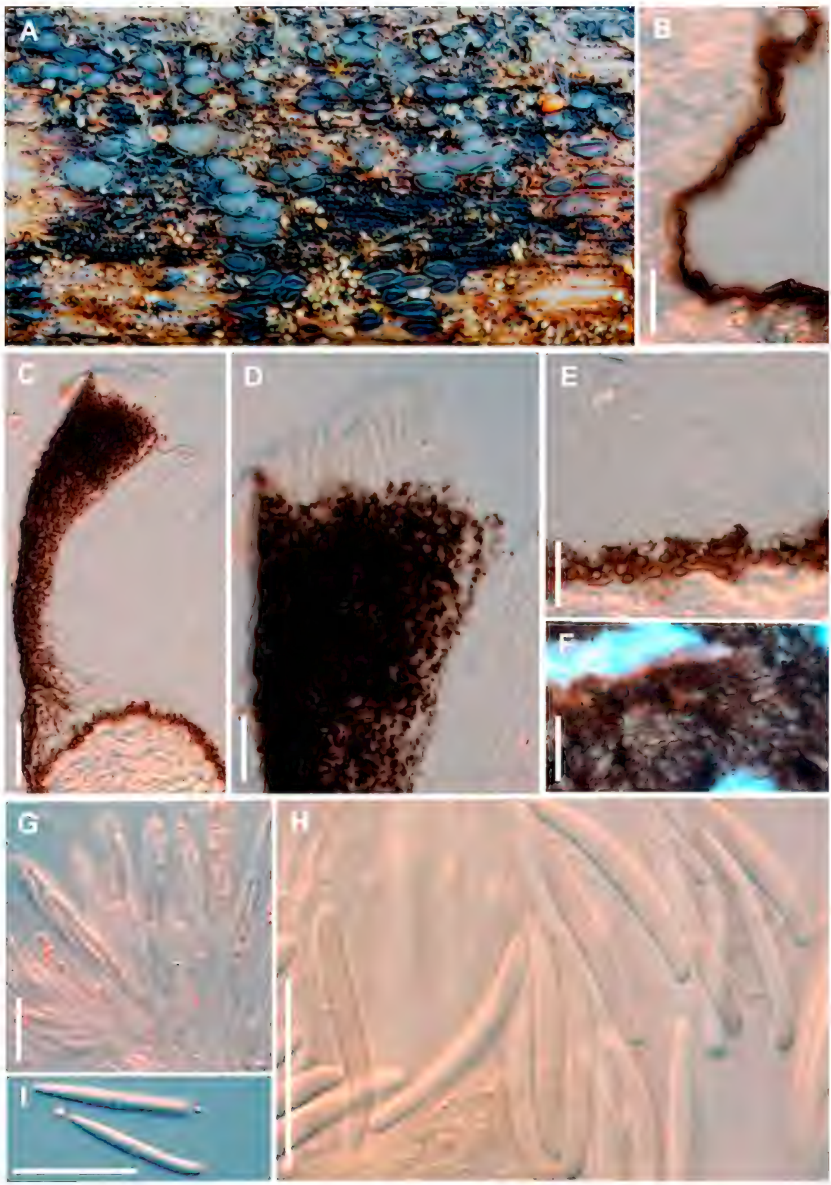

Plate 1. *Hypoderma siculum* (Holotype, K(M) 167515). A. fresh ascomata in situ; B. stromatic tissue surrounding ascomata, vertical section; C. upper wall of ascoma, vertical section; D. detail of lip cells, vertical section; E. detail of lower wall of ascoma, vertical section; F. detail of lower wall of ascoma, squash mount; G. asci and ascospores; H. ascospores in KOH; I. ascospores in water showing gelatinous sheath. Scale bars: A = 1 mm; B, D–H = 20 μm; C = 50 μm.

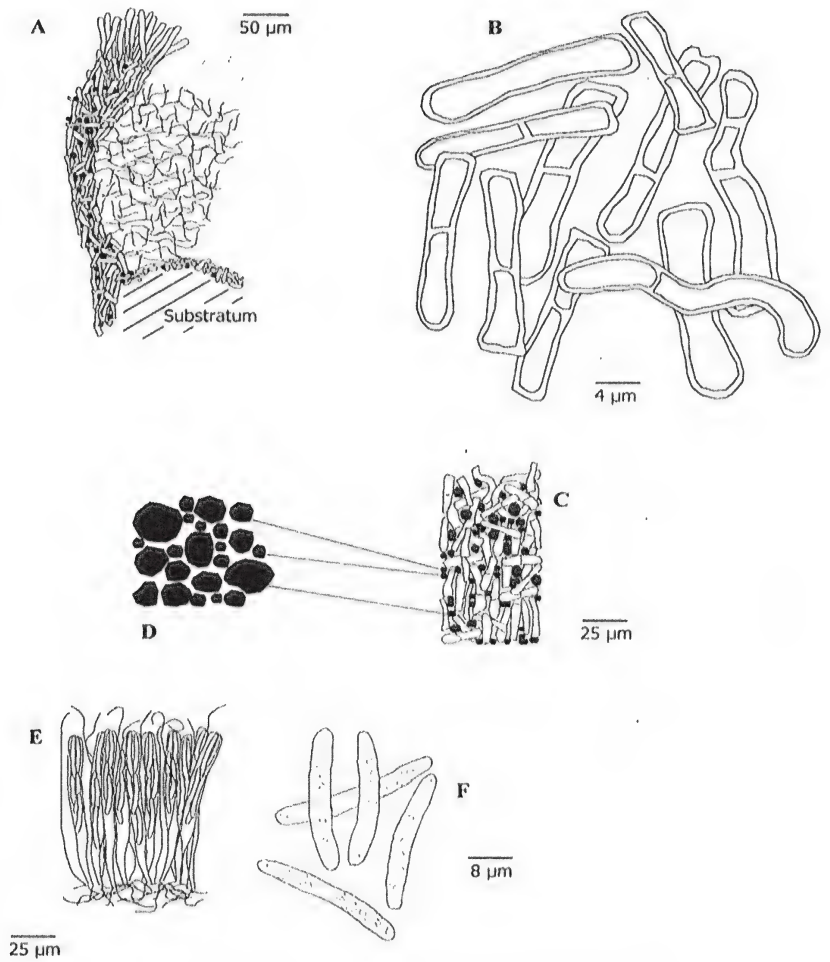

Plate 2. *Hypoderma siculum* (Holotype, K(M) 167515).
A. margin of ascoma in vertical section; B. stromatal cells; C. section of stroma; D. granulose matter; E. asci and ascospores; F. ascospores.

biology supports recognizing the fungus as an independent species. Other morphologically distinct taxa within this clade, such as *Hypoderma cordylines*, are also poorly resolved. More intensive, multi-gene phylogenies of Australasian species have shown that ITS alone does not adequately resolve relationships within this clade (unpublished data). The weak quality of the DNA extracted from the *H. siculum* dried specimens did not permit reliable sequencing of single copy genes.

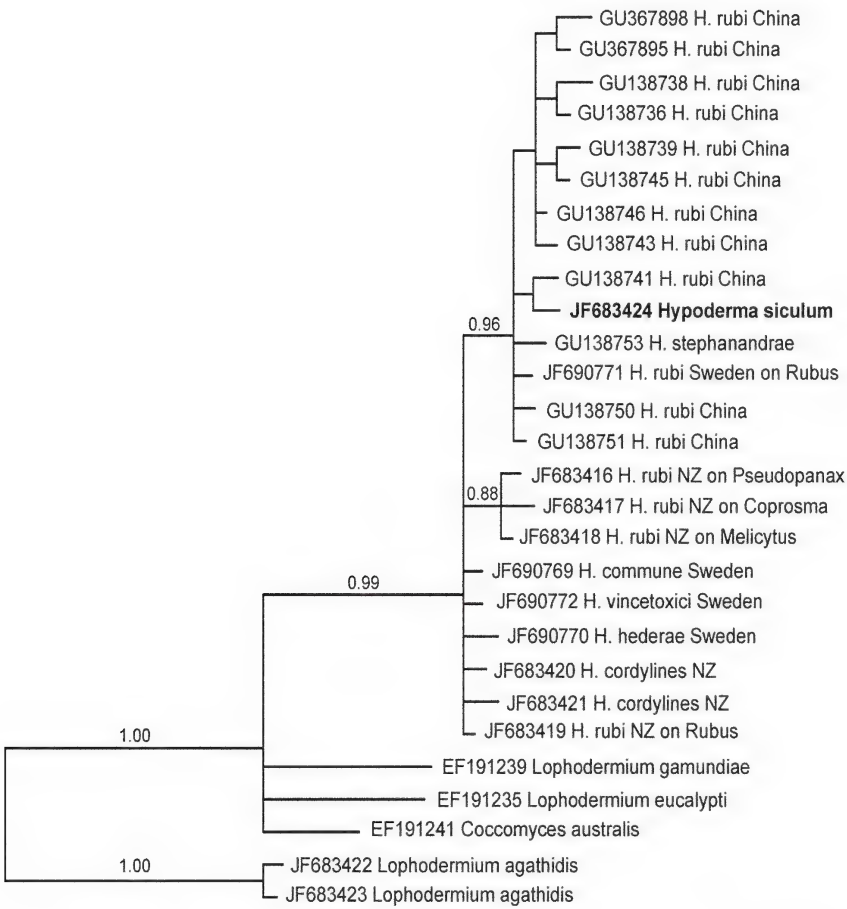

FIGURE 3. 50% majority-rule consensus phylogenetic tree based on Bayesian analysis of *Hypoderma siculum* and related taxa derived from ITS gene sequences. Bayesian posterior probabilities greater than 90% are shown above the edges. *Sclerotinia sclerotiorum* was selected as the outgroup.

Ferula communis L. (*Apiaceae*), a typical herbaceous plant of the Mediterranean area, is commonly associated with two *Hypoderma* species, *H. ferulae* Lantieri (Lantieri 2009) and *H. siculum*. Early reports of *Hypoderma rubi* (Pers.) DC. and *H. commune* (Fr.) Duby on *F. communis* (Duby 1862; Petrak 1943) are difficult to assess, as neither report is supported by specimens. It is uncertain in what sense the authors were using these names and impossible to check whether the specimens they reported match our *Ferula*-specialised species.

Extensive collecting in Sicily revealed only *H. ferulae* and *H. siculum* on *Ferula*. Scalia (1900: 37) reported *H. commune* from Sicily on a species in another genus of *Apiaceae*, *Thapsia garganica* L.; the report included no description of the fungus, and again there is no material available to verify the record. There is no evidence from collections made in Sicily that *H. siculum* occurs on other genera of *Apiaceae* (unpubl. data).

Hypoderma siculum is distinguished from *H. ferulae* by ascospore size and septation (21–24 × 2.5–3 µm and often 1–septate in *H. ferulae*), by the presence of an extended area of stromatic subcuticular tissue surrounding the ascomata, the colour of the hymenium when fresh (yellow-honey with greenish tinges in *H. ferulae*), and the lack of an excipular layer.

Hypoderma siculum resembles *H. rubi* morphologically, but as noted in the phylogenetic section, the application of this name by different authors is uncertain, and genetic relationships amongst specimens identified as *H. rubi* are poorly resolved. *Hypoderma rubi* sensu Powell (1974), Johnston (1990), and Medardi (2006) differs slightly from *H. siculum* in its ascospore size (20–25(–26.5) × 3–4 µm) and the fusiform-navicular ascospore shape. *Hypoderma commune* differs in having ascomata with yellow-greenish hymenium and smaller, non-septate spores (17–20 × 3–4 µm; Ellis & Ellis 1988).

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Tuber sinoalbidum and *T. polyspermum* — new species from China

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Abstract — Two new *Tuber* species from China are described and illustrated. *Tuber sinoalbidum* is recognized by its white mid to large sized ascocarps and spinulose-reticulate ascospores, while *T. polyspermum* is characterized by *Tuber lyonii*-like ascospores and smaller brown ascomata. A molecular study based on the ITS sequences supports the erection of the two new species.

Key words — *Ascomycota*, *Tuberaceae*, truffle

Introduction

Tuber is a genus of ascomycetous fungi that form ectomycorrhizae with shrubs and trees. Species of the genus have been extensively studied because many form edible hypogeous ascocarps — the truffles. Some of these are renowned for their flavor and are commercially important, with the two European species, *T. magnatum* Picco and *T. melanosporum* Vittad., commanding the highest prices. Other species harvested include *T. aestivum* Vittad., *T. borchii* Vittad., *T. gibbosum* Harkn., and *T. indicum* Cooke & Massee (Hall et al. 2007). Over the past two decades the harvest and sale of the Chinese black truffle, *T. indicum*, has increased dramatically and has become a significant source of income for the local farmers who live in the mountainous truffle-producing regions. The two new *Tuber* species we describe here are based on specimens collected by these local farmers.

Materials and methods

Morphological studies

Fresh fruiting bodies were collected from Kunming, Yunnan and Panzhihua City, Sichuan and deposited in BJTC (Herbarium Biology Department, Capital Normal

Table 1. Specimens used in molecular studies and GenBank accession numbers.

Species	Voucher specimen	ITS
Tuber aestivum		AF516788
		AY226042
Tuber huidongense	BJTC FAN104	JF9261163
		DQ486032
Tuber lyonii		EU394704
		EU268568
Tuber polyspermum	BJTC FAN131	JF9261165
Tuber rufum Picco		DQ329375
		FM205665
Tuber sinoalbidum	BJTC FAN105	JF9261164
Tuber spinoreticulatum Uecker & Burds.		FJ80988
		FJ74891
Tuber taiyuanense B. Liu		GU979033
		DQ478662
Tuber umbilicatum Juan Chen & P.G. Liu		FJ797880
		FJ797879
		GU979031

University). The macroscopic characteristics were described from both fresh and rehydrated specimens. The microscopic characteristics are described from razor-blade sections of the specimens mounted in 3% KOH, Melzer's reagent, or cotton blue. For scanning electron microscopy (SEM), ascospores were scraped from dried gleba of the fruit bodies and mounted in distilled water on a cover glass. After air drying the cover glasses were directly attached to a SEM stub with doubled-sided tape, and then coated with gold-palladium. The treated materials were examined and photographed with a Hitachi S-4800 SEM.

Molecular methods

Herbarium samples were crushed by shaking for 3 min at 30 Hz (Mixer Mill MM 301, Retsch, Haan, Germany) in a 1.5 ml tube together with one 3 mm in diameter tungsten carbide ball. Total genomic DNA was then extracted using the PeqLab E.Z.N.A._Fungal DNA kit following the manufacturer's protocol. The ITS region was amplified with PCR using the primers ITS1/ITS4 (White et al. 1990). PCR was performed in 50 μl reactions containing DNA template 2 μl, primer (10 μM/L) 2 μl each, 2× Master Mix (Tiangen Biotech (Beijing) Co. Ltd.) 25 μl. PCR reactions were run as follows: an initial denaturation at 95 °C for 3 min, followed by 30 cycles at 95 °C for 2 min, 55 °C for 25 s, 72 °C for 2 min, and a final extension at 72 °C for 10 min. The PCR products were sent to Invitrogen Biotechnology Co. Ltd. (Beijing, China) for purifying, sequencing, and editing. The other sequence data of ITS rDNA included in this study were downloaded from GenBank. GenBank numbers are shown in Table 1.

Phylogenetic analyses

DNA sequences were aligned with Clustal X (Thompson et al. 1997). The alignment was manually adjusted with Se-Al v.2.03a (Rambaut 2000). The aligned dataset was analyzed with maximum parsimony (MP) using PAUP*4.0b10 (Swofford 2002).

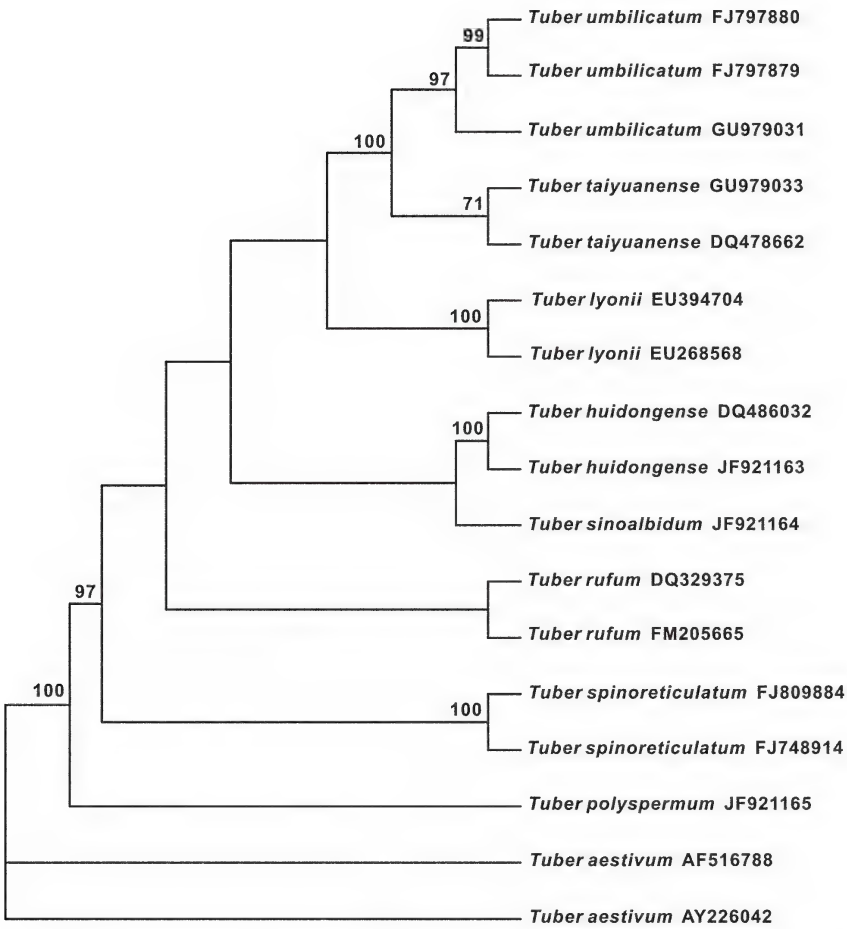


FIG. 1. Phylogeny derived from maximum parsimony analysis of the ITS rDNA sequences of some *Tuber* species with spiny and spinulose-reticulate ornamentation on the ascospore surface, using *T. aestivum* as outgroups. Bootstrap values of more than 70% from 1000 replications are shown above the respective branches.

Maximum parsimony analysis was conducted using heuristic searches with 1000 replicates of random-addition sequence, tree bisection reconnection (TBR) branch swapping algorithm. All characters were equally weighted and unordered. Gaps were treated as missing data to minimize homology assumptions. A bootstrap (BS) analysis was performed with 1000 replicates, each with 10 random taxon addition sequences. TBR branch swapping was employed.

Results

Molecular phylogenetics

The maximum parsimony analysis of sequences resulted in one most parsimonious tree (Fig. 1) with a length (TL) = 1082 steps, consistency index (CI) = 0.7126, retention index (RI) = 0.7347, homoplasy index (HI) = 0.2874, and rescaled consistency index (RC) = 0.5731.

Phylogenetic analyses of ITS sequences revealed that all cited species with light colored ascomata and spiny and spinulose-reticulate ascospore ornamentations grouped together with a bootstrap support value of 100%. *Tuber polyspermum* was placed as a distinct clade in the genus. The sequence of *T. sinoalbidum* and two sequences of *T. huidongense* were grouped in a clade but with a lower BS support.

Taxonomy

Tuber polyspermum L. Fan & C.L. Hou, **sp. nov.** Figs. 2–7

MycoBank MB 519840

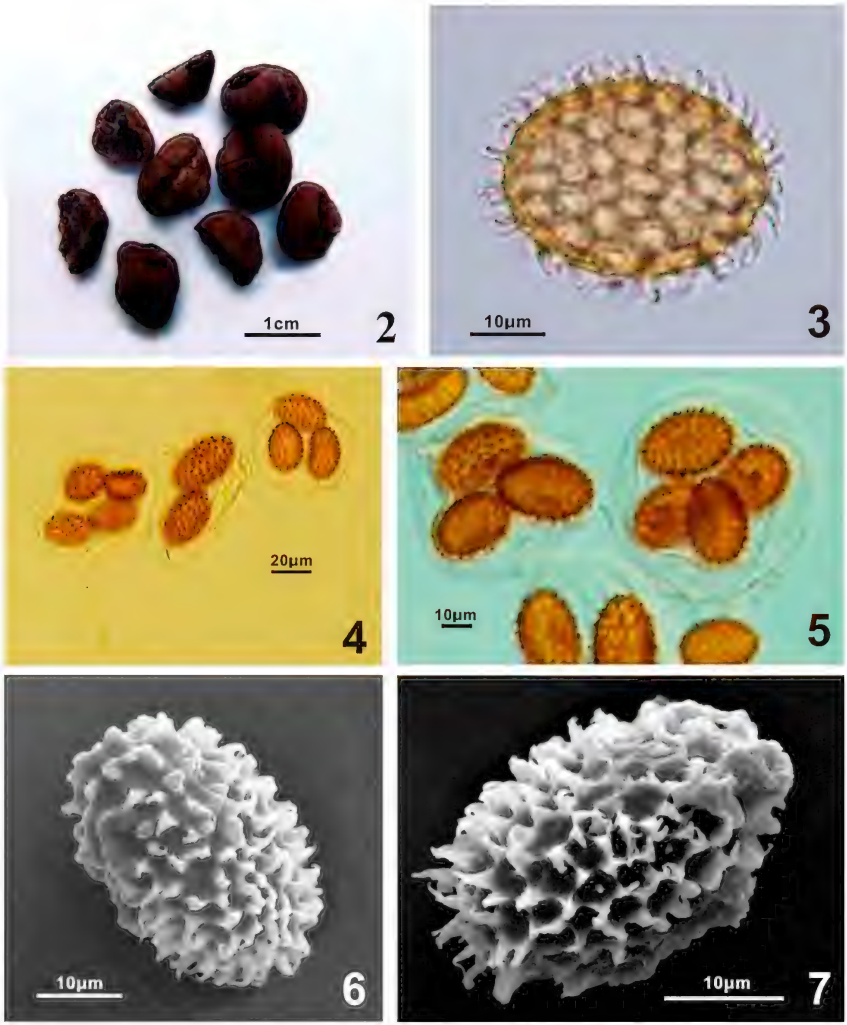
Ascomata brunnea vel griseo-brunnea, 0.5–1.5 cm diam, glabra vel subglabra, basi umbilicata. Peridium 150–200 μm crassum, strato exteriore pseudoparenchymato et strato interiore ex hyphis intricatis instructum. Gleba solida, brunnea vel purpureo-brunnea, venis albis. Asci 65–85 × 45–60 μm, 1–4(–5) spori. Ascosporae ellipsoideae, brunneae vel flavo-brunneae, 25–37.5(–45)× 20–25(–30) μm, spineae vel spineo-reticulatae.

Type: China. Yunnan Province, Kunming, hypogeous, under soil of *Pinus yunnanensis* Franch., 20 Dec. 2002, Jin-zhong Cao 2002112001 (**Holotype**, BJTC FAN131).

Etymology: *polyspermum* (Lat.) refers to the large number of ascospores in the ascomata.

Ascomata subglobose, 0.5–1.5 cm in diam., usually with an umbilicate depression at the base, brown to gray-brown when fresh, surface smooth. Odor pungent when fresh. Peridium 150–200 μm in thickness, composed of two layers; outer layer 50–100 μm thick, pseudoparenchymatous, composed of subglobose or subangular, light brown cells 7.5–15(–20) μm in diam.; inner layer 100–150 μm thick, textura intricate, composed of interwoven hyphae with hyaline, thin-walled cells 2.5–5 μm broad. Gleba brown or purple brown at maturity, veins white, large and rare, containing a great number of spores. Asci globose to subglobose, 65–85 × 45–60 μm, sessile or with a short stalk, 1–4 (–5) spored. Ascospores ellipsoid, hyaline at first, brown or yellow brown at maturity, short spiny or spinulose-reticulate, 25–37.5 (–45) × 20–25 (–30) μm excluding the ornamentation, ornamentations up to 2.5–3 μm high, meshes closed or unclosed, regular to irregular, 5–8 across the spore width.

Comments — *Tuber polyspermum* is almost indistinguishable from the American species *T. lyonii* Butters in both macro- and micro-morphological characteristics except for small differences in the shape of the ascomata (more



Figs 2–7. *Tuber polyspermum* (BJTC FAN131, holotype). 2. Ascocarps. 3. Ascospore observed under the light microscope. 4–5. Asci and ascospores observed under the light microscope. 6–7. Ascospore observed under the scanning electronic microscope.

or less umbilicate in *T. polyspermum*) and the color of the gleba (darker in *T. polyspermum*). It is clear that these differences alone are insufficient to erect a new species. However, the molecular analysis showed that the Chinese material did not group in the same clade with *T. lyonii*, and instead was in a clade of its own (Fig. 1). Because of this and the clear geographic separation between Asia

and North America, we here treat the Chinese material as a new species that is similar to, but distinct from, *T. lyonii*.

A very interesting characteristic of *T. polyspermum* is the very large number of ascospores in the mature ascomata in the type specimen, especially in the dried specimens, when compared with other *Tuber* species. The specific epithet "*polyspermum*" refers to this.

Tuber sinoalbidum L. Fan & J.Z. Cao, **sp. nov.** FIGS. 8–12

MycoBank MB 519841

Ascomata albida, 2–4.5 cm diam., subglobosa vel globosa. Peridium 200–250 µm crassum, strato exteriore pseudoparenchymato et strato interiore ex hyphis intricatis instructum. Gleba solida, griseo-albida, veins albis. Asci 45–60 × 60–80 µm, 1–4(–5) spori. Ascosporae ellipsoideae, brunneae vel flavo-brunneae, 25–37.5(–45) × 17.5–25(–30) µm, spineo-reticulatae.

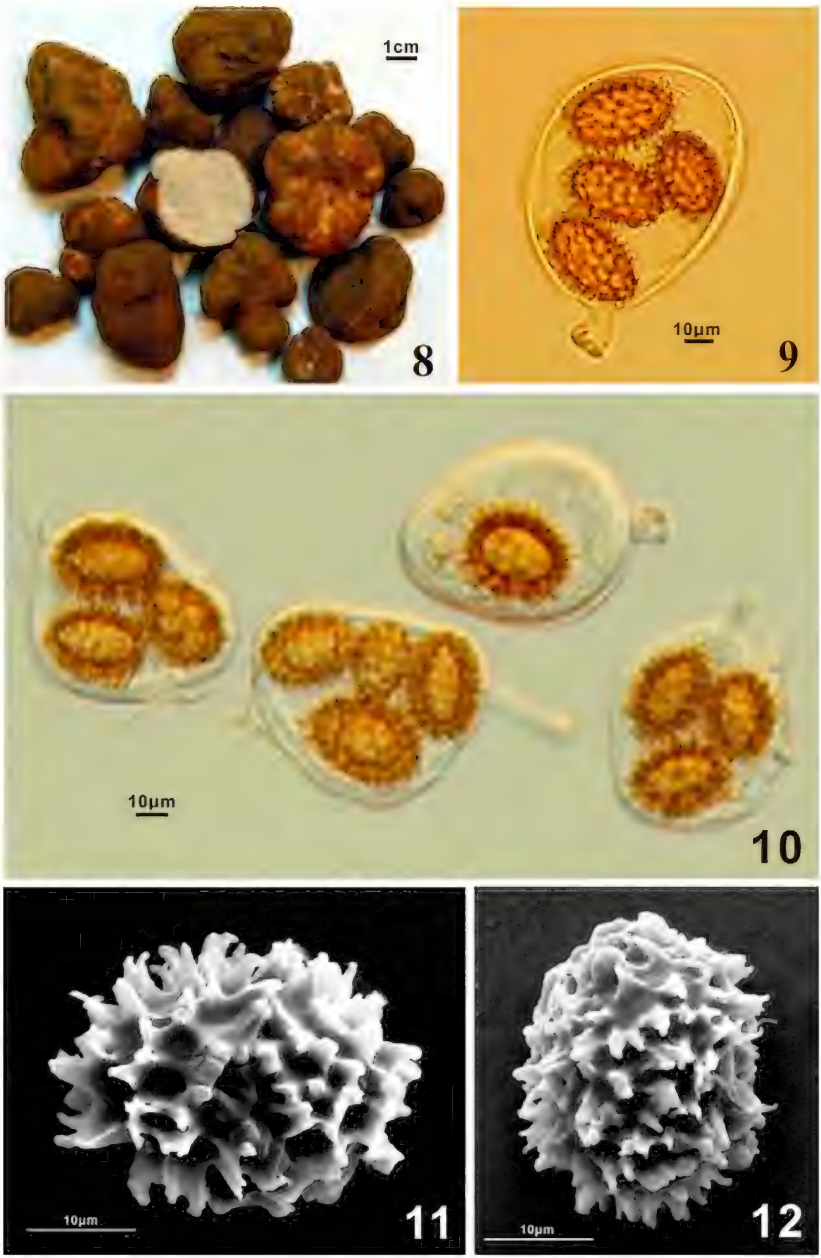
TYPE: China. Sichuan Province, Panzhihua City, hypogeous, under soil in forest, 31 Dec. 2007, De-fu Liu (**Holotype**, BJTC FAN105).

ETYMOLOGY: *sinoalbidum* (Lat.) refers to a whitish *Tuber* species from China.

ASCOMATA hypogeous, globose or subglobose, 2–4.5 cm in diam., white to whitish when fresh, surface smooth to very fine verrucose. Odor light. PERIDIUM 200–250 µm in thickness, composed of two layers; outer layer 50–100 µm thick, pseudoparenchymatous, composed of subglobose or subangular light brown colored cells 7.5–12.5 µm in diam.; inner layer 150–200 µm thick, textura intricate, composed of interwoven hyphae with hyaline, thin-walled cells 2.5–5 µm broad. GLEBA white, pale white or grey white at maturity, veins white at first and light brown at maturity, narrow and numerous. ASCI globose or subglobose, 45–60 × 60–80 µm excluding the stalk, stalk 8–20 × 3–5 µm long, 1–4(–5) spored. ASCOSPORES ellipsoid to broad ellipsoid, hyaline at first, brown or yellow brown at maturity, spinulose-reticulate, 25–37.5(–45) × 17.5–25(–30) µm excluding the ornamentation, ornamentations up to 3.5–5(–7.5) µm high, meshes 4–6 across the spore width.

ADDITIONAL SPECIMEN EXAMINED: **CHINA. YUNNAN PROVINCE**, Kunming, from market, 17 Dec. 2005, Jin-zhong Cao (BJTC FAN101).

COMMENTS —*Tuber huidongense* Y. Wang, a common endemic species in Sichuan and Yunnan (Deng et al. 2009), is similar to *T. sinoalbidum* in ascospore ornamentation, but differs in having brown ascomata when fresh, and a blackish colored gleba at maturity. Also *T. huidongense* ascomata are normally small, rather than medium to large as in *T. sinoalbidum*. The phylogenetic analysis (FIG. 1) also shows that while *T. huidongense* groups in a clade with *T. sinoalbidum*, the bootstrap support value is low. This indicates they are closely related but clearly separate. Therefore, we conclude that *T. sinoalbidum* is a distinct species.



Figs 8–12. *Tuber sinoalbidum* (BJTC FAN105, holotype). 8. Ascocarps. 9–10. Asci and ascospores observed under the light microscope. 11–12. Ascospore observed under the scanning electronic microscope.

The color of *T. sinoalbidum* when fresh is similar to that of *T. magnatum* and *T. latisporum* Juan Chen & P.G. Liu, but they can be separated easily from the new species by the typical reticulate ascospore ornamentations in both *T. magnatum* and *T. latisporum* (Riousset et al. 2001; Chen & Liu 2007).

Acknowledgments

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Hymenochaete in China. 2. A new species and three new records from Yunnan Province

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Abstract — A new species and three new Chinese records of *Hymenochaete* are reported: *H. yunnanensis* sp. nov., *H. fulva*, *H. spathulata*, and *H. sphaerospora*. They were all collected from Caiyanghe Nature Reserve, Yunnan Province, southwestern China. *Hymenochaete yunnanensis* (in sect. *Hymenochaete*) is characterized by setal hyphae and heavily encrusted hyphae and hymenial cells. Complete descriptions with illustrations are provided for the four species.

Key words — *Hymenochaetaceae*, taxonomy, wood-inhabiting fungi

Introduction

Because of the abundant woody plants and the complex topography, the diversity of wood-inhabiting fungi is extremely high in Yunnan Province and adjacent areas of southwestern China. During the last decade, the porioid wood-inhabiting fungi in these areas have been intensively investigated and many papers were published (Cui et al. 2011; Dai & Zhou 2000, Dai et al. 2007, Dai 2010, 2011a; Niemelä et al. 2001; Yu et al. 2008; Yuan & Dai 2008a,b). However, the corticioid wood-inhabiting fungi in these areas have not been sufficiently studied. The corticioid genus, *Hymenochaete* Lév., is one of the most important genera in the *Hymenochaetaceae*. Although it has been studied by several mycologists (Dai 2010, 2011b; Maekawa & Zang 1995; Xu et al. 2003; Zhang & Dai 2005), the genus has not yet been systematically studied in southwestern China.

Caiyanghe Natural Reserve, located in southern Yunnan Province, is one of the famous nature reserves in China because it is a transitional area from the tropical to the subtropical zone with the altitude ranging from 980–1707 m. The main vegetation type is a monsoon evergreen broadleaf forest, comprising

mainly species of *Castanopsis*, *Lithocarpus* and *Schima* (Jing & Chen 2007). In the summer of 2011, an intensive survey of wood-inhabiting fungi in Caiyanghe and Xishuangbannan Natural Reserve, southern Yunnan Province, was carried out and more than 130 *Hymenochaete* specimens were collected. Among these specimens, *Hymenochaete yunnanensis* is identified as a new species. In addition, *H. fulva*, *H. spathulata* and *H. sphaerospora* are found in China for the first time. Illustrated descriptions of these species are provided in this paper.

Materials & methods

Voucher specimens are deposited in the herbarium of Beijing Forestry University (BJFC), and the microscopic procedure follows He (2010). In the text the following abbreviations are used: L = mean spore length (arithmetical average of all spores), W = mean spore width (arithmetical average of all spores), Q = variation in the L/W ratios between the specimens studied (quotient of the mean spore length and the mean spore width of each specimen), n = the number of spores measured from given number of specimens. In presenting the size range of spores, 5% of the measurements were excluded from each end of the range, and the measurements were given in parentheses. IKI stands for Melzer's reagent, KOH for 5% potassium hydroxide, and CB is the abbreviation of Cotton Blue. IKI– = inamyloid and nondextrinoid, CB– = acyanophilous. Special color terms follow Petersen (1996).

Taxonomy

Hymenochaete yunnanensis S.H. He & Hai J. Li, **sp. nov.** FIGS. 1–2

MycoBank MB 563149

Carpophorum effusum vel effuso-reflexum, laxe adnatum. Cortex, tomentum et stratum hypharum adsunt. Hymeniis et hyphae cum resinaceus granulum encrustatae. Setosum hyphae adsunt. Setae 40–75(–80) × 6–7(–9) µms, porae ellipsoideae, 5–6.5(–6.8) × (2.8–)3–3.5(–3.8) µm.

TYPE: China. Yunnan Prov., Pu'er, Caiyanghe Nat. Res., alt ca. 1200 m, on fallen angiosperm branch, 9 VI 2011 He 709 (**holotype**, BJFC).

ETYMOLOGY: *yunnanensis*, refers to its type locality of Yunnan Province, China.

FRUITBODY: Annual, effused or effused-reflexed with slightly elevated margins, loosely adnate, easily detached, coriaceous, brittle when dry, first as small colonies, later confluent, resupinate part up to 8 cm long or more in longest dimension, reflexed part projecting up to 0.3 cm, 180–300 µm thick in section. Pileal surface rust-brown to dark brown, silky, tomentose, curving down when dry. Hymenophore smooth or with scattered tubercles, grayish brown to clay-buff, usually covered with many yellowish resinous matters, not cracked or with few deep crevices when dry; resupinate margin thinning out, distinct, silky, fimbriate, yellowish to yellowish brown when juvenile, becoming indistinct or slightly elevated, concolorous with hymenium when mature.

Fig. 1. Basidiocarps of *Hymenochaete yunnanensis* (He 709, holotype).

Hyphal structure: Hyphal system subdimitic; generative hyphae without clamp connections; tissue darkening but otherwise unchanged in KOH.

Subiculum: Cortex, tomentum and hyphal layer present. Cortex composed of strongly agglutinated hyphae, 20–50 μm thick. Generative hyphae hyaline to yellowish brown, thin- to thick-walled, septate, moderately branched, sometimes collapsed, more or less interwoven and agglutinated, usually heavily encrusted with resinous matters, 2–4 μm in diam. Skeletoids with distinctly thickened walls, reddish brown, rarely septate and branched. Setal hyphae (embedded setae) frequently present, up to 200 μm long.

Stratified hymenium: Hyphae in this layer similar to those in subiculum, hyaline, thick-walled, agglutinated, uprightly arranged, 2.5–4.5 μm in diam. Setal layer thickening, composed of several rows of overlapping setae. Setae numerous, subulate or fusiform, reddish brown, with an acute tip, projecting up to 45 μm above the hymenium, 40–75(–80) × 6–7(–9) μm. Hymenial cells usually heavily encrusted with resinous matters. Cystidia and hyphidia absent. Basidia clavate, with four sterigmata and a simple septum at base, 12–20 × 3.8–5 μm; basidioles in shape similar to basidia, but slightly smaller.

Basidiospores ellipsoid, some with tapering apex, hyaline, thin-walled, smooth, IKI–, CB–, 5–6.5(–6.8) × (2.8–)3–3.5(–3.8) μm, L = 5.79 μm, W = 3.16 μm, Q= 1.79–1.87 (n = 60/2).

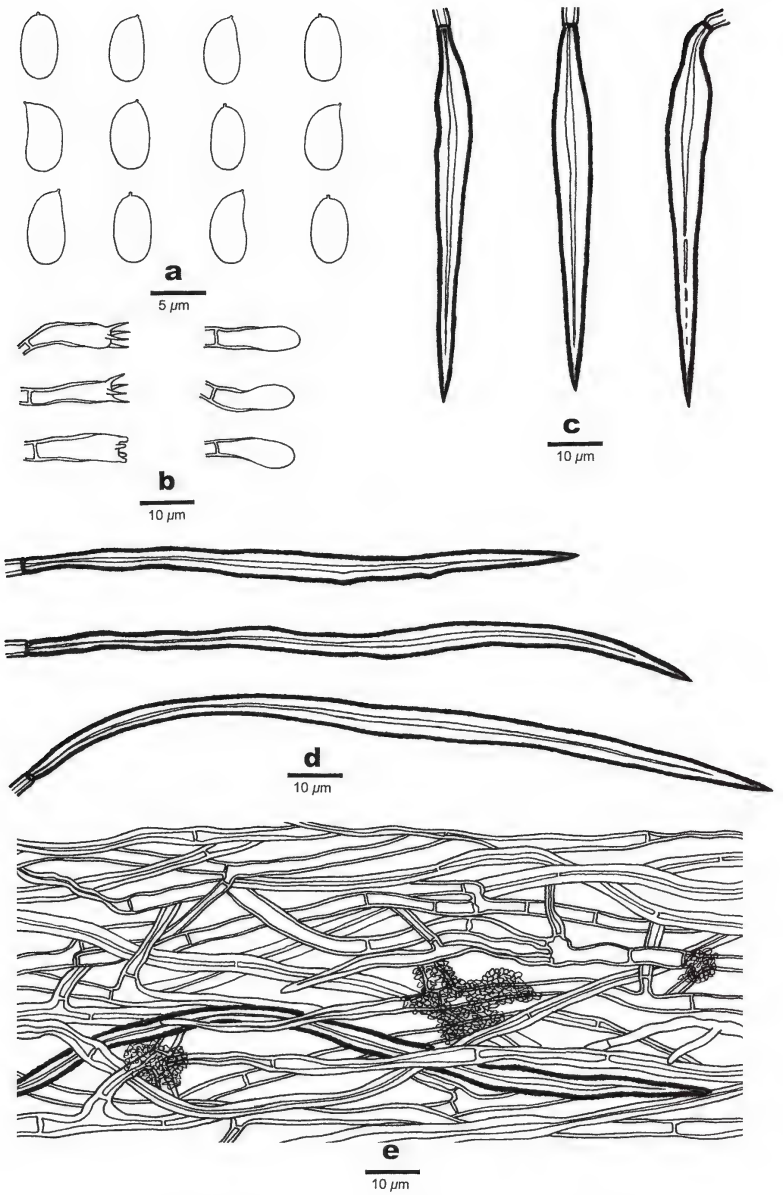

FIG. 2. Microscopic structures of *Hymenochaete yunnanensis* (drawn from the holotype). a: Basidiospores. b: Basidia and basidioles. c: Setae. d: Setal hyphae. E: Hyphae from subiculum.

Additional specimens examined: **CHINA. Yunnan Prov.**, Pu'er, Caiyanghe Nat. Res., alt ca. 1200 m, on fallen angiosperm twig, 9 VI 2011 He 690 (BJFC).

Remarks: *Hymenochaete yunnanensis* belongs to sect. *Hymenochaete* (presence of cortex, hyphal layer and setal layer; Léger 1998) and is characterized by the presence of setal hyphae and the heavily encrusted hyphae and hymenial cells. Usually the hymenial cells are so heavily encrusted that the resinous compounds form numerous yellowish dots on the hymenophore surface.

Microscopically, the new species is very close to *H. ulmicola* Corfixen & Parmasto; however, *H. ulmicola* differs in growing in the bark fissures of old living *Ulmus* trees and in having thicker, harder and smaller basidiocarps and slightly larger basidiospores (5.5–7.5 × 3–4 μm; Corfixen & Parmasto 2005). Another similar species, *H. colliculosa* (Sacc.) Parmasto, can be distinguished from the new species by its larger setae (80–110 × 7.5–11 μm) and basidiospores (5.5–7.5 × 3.6–4.8 μm; Parmasto 2005). *Hymenochaete fulva* also has encrusted hyphae, but differs from *H. yunnanensis* in the yellowish brown basidiocarps, broadly ellipsoid basidiospores (5–6 × 3.2–4 μm), and absence of setal hyphae (Parmasto 2001).

Hymenochaete fulva Burt, Ann. Mo. Bot. Gard. 5: 354, 1918 Figs. 3–4

Fruitbody: Annual, effused, closely adnate, coriaceous, first as small colonies, later confluent up to 8 cm or more in longest dimension, 150–300 μm thick. Hymenophore smooth, cinnamon-brown, yellowish brown or clay-

Fig. 3. A basidiocarp of *Hymenochaete fulva* (He 620).

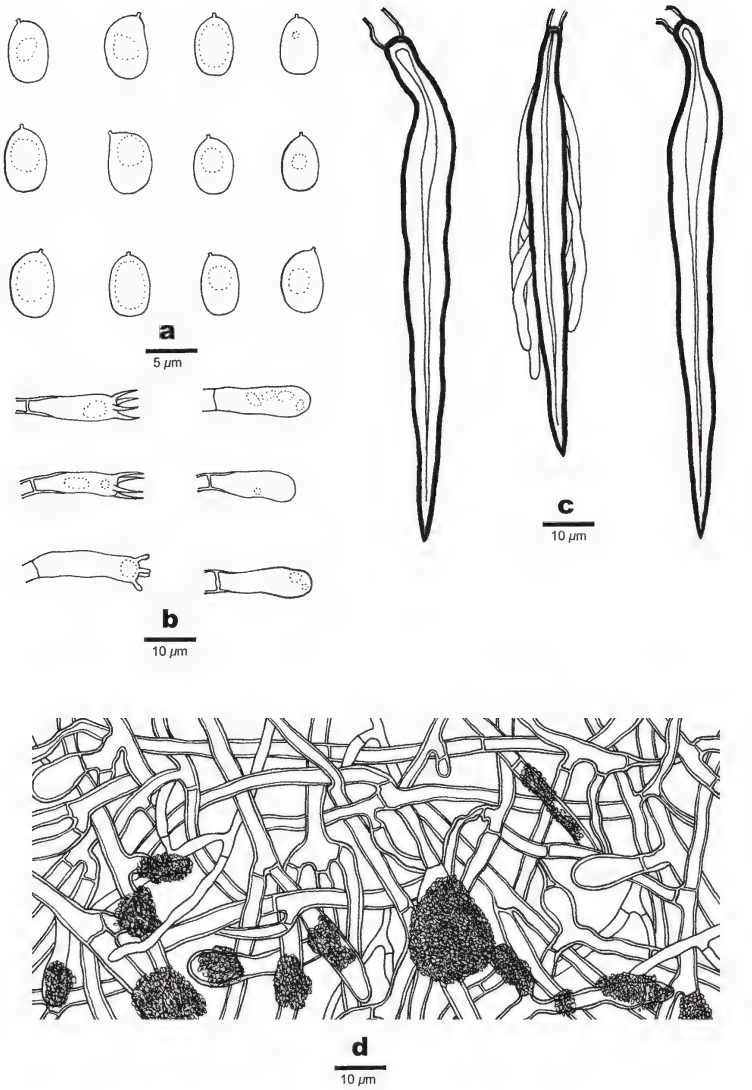

Fig. 4. Microscopic structures of *Hymenochaete fulva* (drawn from He 620).
a: Basidiospores. b: Basidia and basidioles. c: Setae. d: Hyphae from subiculum.

buff, azonate, not cracked or sometimes with numerous deep crevices; margin thinning out, distinct, paler than hymenophore surface, yellowish brown.

Hyphal structure: Hyphal system monomitic; generative hyphae without clamp connections; tissue darkening but otherwise unchanged in KOH.

Subiculum: Tomentum absent; cortex and hyphal layer present. Cortex composed of strongly agglutinated hyphae, 10–20 µm thick. Generative hyphae hyaline to yellowish brown, thick-walled with a wide lumen, some lightly or heavily encrusted with yellowish brown resinous granules, frequently septate, moderately branched, loosely interwoven, 2.5–5 µm in diam.

Stratified hymenium: Hyphae in this layer similar to those in subiculum, yellowish to yellowish brown, thick-walled, more or less agglutinated, interwoven, 2–4.8 µm in diam. Crystals occasionally present in the hymenium. Setal layer composed of 1–3 rows of overlapping setae. Setae numerous, reddish brown, subulate, sometimes enmeshed with a hyphal sheath, with an acute tip, projecting up to 80 µm above the hymenium, 60–100(–110) × 7–10(–11) µm. Hyphidia absent, encrusted cystidia-like hyphal ends present. Basidia clavate, with four sterigmata and a simple septum at base, 15–22 × 4–5 µm; basidioles in shape similar to basidia, but slightly smaller.

Spores: Basidiospores broadly ellipsoid, hyaline, thin-walled, smooth, usually bearing a large guttule, IKI–, CB–, (4.8–)5–6 × 3.5–4 µm, L = 5.43 µm, W = 3.82 µm, Q = 1.40–1.44 (n = 60/2).

Specimens examined: **China. Yunnan Prov.**, Pu'er, Caiyanghe Nat. Res., alt ca. 1400 m, on fallen angiosperm twig, 6 VI 2011 He 620 & 640.

Remarks: *Hymenochaete fulva* belongs to sect. *Hymenochaete* and is characterized by its yellowish brown basidiocarps, encrusted hyphae, and broadly ellipsoid basidiospores. The "cystidia" of the species cited by Burt (1918) and Léger (1998) are actually encrusted hyphal ends (Parmasto 2001). *Hymenochaete fulva* has also been reported in Mexico and Jamaica (Parmasto 2001). The species is close to *H. rhododendricola* S.H. He & Hai J. Li and *H. rhabarbarina* (Berk.) Cooke; however, both species differ from *H. fulva* in their encrusted setae and absence of cortices (He & Li 2011a; Parmasto 2001).

Hymenochaete spathulata J.C. Léger, Bull. Soc. Mycol. Fr. 96: 409, 1981 ["1980"] Figs. 5–6

Fruitbody: Annual, effused, closely adnate, coriaceous, first as small colonies, later confluent up to 20 cm or more in longest dimension, 180–280 µm thick. Hymenophore smooth, pale mouse-gray to light vinaceous gray, azonate, not cracked; margin thinning out, distinct, whitish, fimbriate when juvenile, becoming indistinct, concolorous with hymenophore surface when mature.

Hyphal structure: Hyphal system monomitic; generative hyphae without clamp connections; tissue darkening but otherwise unchanged in KOH.

FIG. 5. Basidiocarps of *Hymenochaete spathulata* (He 685).

SUBICULUM: Tomentum and hyphal layer absent. Cortex locally present, thin, composed of strongly agglutinated hyphae.

STRATIFIED HYMENIUM: Generative hyphae hyaline to yellowish brown, thin- to thick-walled with a wide lumen, densely interwoven, 1.8–3.6 µm in diam. Setal layer thickening, composed of several rows of overlapping setae. Setae scattered, reddish brown, spathulate, with an obtuse tip, usually lightly encrusted with crystals at the tip, projecting up to 50 µm above the hymenium, 65–100 × 8–13 µm. Cystidia and hyphidia absent. Basidia clavate, with four sterigmata and a simple septum at base, 12–16 × 3.5–4 µm; basidioles in shape similar to basidia, but slightly smaller.

SPORES: Basidiospores cylindrical to allantoid, hyaline, thin-walled, smooth, IKI–, CB–, 6–7.1(–7.5) × 1.8–2.1 µm, L = 6.65 µm, W = 1.98 µm, Q = 3.36 (n = 30/1).

SPECIMENS EXAMINED: **CHINA. YUNNAN PROV.**, Pu'er, Caiyanghe Nat. Res., alt ca. 1200 m, on fallen angiosperm twig, 9 VI 2011 He 685 & 704.

REMARKS: *Hymenochaete spathulata* is distinguished in the genus by its gray basidiocarps, spathulate setae, and cylindrical to allantoid basidiospores (Léger 1998). Another species with round-tipped setae is *H. ryvardenii* Parmasto, which differs from *H. spathulata* by thicker (≤ 800 µm) basidiocarps and larger ellipsoid basidiospores (6.5–8 × 3–3.6 µm; Parmasto 2000). Previously

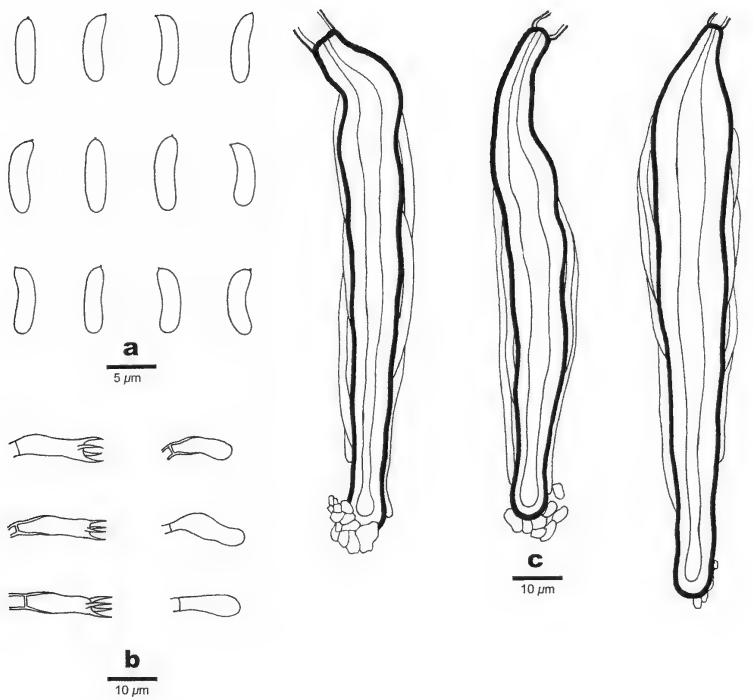

Fig. 6. Microscopic structures of *Hymenochaete spathulata* (drawn from the He 685). a: Basidiospores. b: Basidia and basidioles. c: Setae.

H. spathulata was known only from Gabon and the Central African Republic (Léger 1998).

Hymenochaete sphaerospora J.C. Léger & Lanq., Bull. Soc. Mycol. Fr. 103: 48, 1987 Figs. 7–8

Fruitbody: Perennial, effused, closely adnate, woody hard when dry, confluent up to 20 cm or more in longest dimension, 250–400 µm thick. Hymenophore smooth, pale mouse-gray to grayish brown, azonate, not cracked or finely cracked when dry; margin thinning out, distinct, fawn to cinnamon, up to 2 mm wide.

Hyphal structure: Hyphal system monomitic; generative hyphae without clamp connections; tissue darkening but otherwise unchanged in KOH.

Subiculum: Tomentum absent. Cortex and hyphal layer sometimes present.

FIG. 7. A basidiocarp of *Hymenochaete sphaerospora* (He 691).

STRATIFIED HYMENIUM: Generative hyphae hyaline to yellowish brown, thick-walled, agglutinated, densely interwoven, 2–3 μm in diam. Setal layer thickening, composed of several rows of overlapping setae. Setae numerous, reddish brown, subulate, encrusted with fine crystals in the upper part, with an acute tip, projecting up to 40 μm above the hymenium, 65–110 × 9–16(–19) μm. Cystidia absent. Simple hyphidia present. Basidia clavate or cylindrical, with four sterigmata and a simple septum at base, 23–34 × 4.5–6 μm; basidioles in shape similar to basidia, but distinctly smaller.

SPORES: Basidiospores broadly ellipsoid or subglobose, hyaline, thin-walled, smooth, usually bearing a large guttule, IKI–, CB–, 5–6 × 4–5 μm, L = 5.52 μm, W = 4.33 μm, Q = 1.27 (n = 30/1).

SPECIMENS EXAMINED: **CHINA. YUNNAN PROV.**, Pu'er, Caiyanghe Nat. Res., alt ca. 1200 m, on fallen angiosperm twig, 9 VI 2011 He 691 & 715.

REMARKS: Léger (1998) and Parmasto (2005) previously reported *H. sphaerospora* from Africa. It is diagnosed by the gray basidiocarps, large encrusted setae, and broadly ellipsoid or subglobose basidiospores. *Hymenochaete macrospora* Y.C. Dai, which has similar basidiospores, differs from *H. sphaerospora* in the brownish annual basidiocarps and smooth setae (Dai et al. 2000). The also similar *H. megaspora* S.H. He & Hai J. Li is distinguished from *H. sphaerospora* by its larger spores (7.5–10 × 5–7) and effused-reflexed basidiocarps (He & Li 2011b).

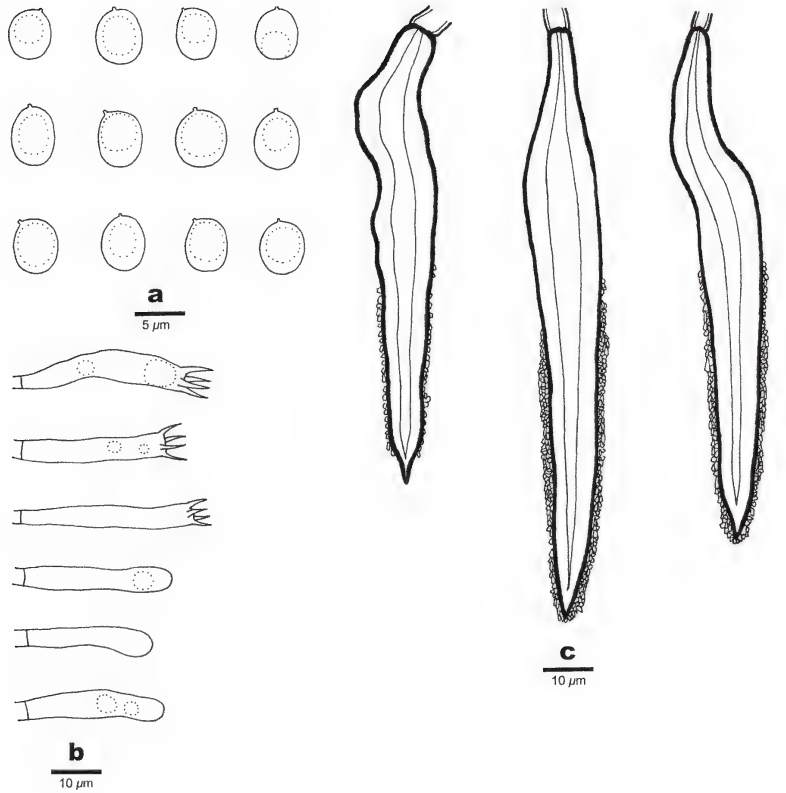

Fig. 8. Microscopic structures of *Hymenochaete sphaerospora* (drawn from He 691). a: Basidiospores. b: Basidia and basidioles. c: Setae.

To date, 19 *Hymenochaete* species (including the four reported here) have been reported from Yunnan Province (Maekawa & Zang 1995; Xu et al. 2003; Zhang & Dai 2005).

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Lichenological notes 3: *Sarcogyne plicata* in California

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Abstract — *Sarcogyne plicata*, a California endemic, is revised and discussed. A taxon referred to in the literature as *Sarcogyne "privigna,"* which looks similar to *S. plicata* and is sympatric in California, is discussed. The name is considered invalid for the taxon.

Key words — *Acarosporaceae*, caliciphile, Joshua Tree National Park, nomenclature

Introduction

The type of *Sarcogyne* Flot. is *S. corrugata* Flot. (= *S. clavus* (DC.) Kremp.) (Jørgensen & Santesson 1993). The genus currently comprises species with lecideine apothecia with an excipulum of melanized (or even carbonized) hyphae that lack any epihymenial accretions as are found in species currently included in *Polysporina* Vězda. The exact number of species in the genus is unknown, partly because the current generic circumscription is paraphyletic (Reeb et al. 2004; Westberg, pers. comm.) and partly because many species need revision or are known only from lost types (Knudsen & Standley 2007; Lendemer et al. 2009b).

In North America we currently recognize 15 described *Sarcogyne* species. Twelve species are known from collections made in the last 15 years: *S. arenosa* (Herre) K. Knudsen & S.M. Standl. (Knudsen & Standley 2007; Lendemer et al. 2009a), *S. clavus* (Magnusson 1935a, b; Harris & Ladd 2005; Knudsen & Standley 2007), *S. crustacea* K. Knudsen & Kocourk. (Knudsen & Kocourková 2010), *S. dakotensis* H. Magn. (Magnusson 1935b; Knudsen & Standley 2007), *S. desolata* (H. Magn.) K. Knudsen & S.M. Standl. (Knudsen & Standley 2007), *S. novomexicana* H. Magn. (Knudsen & Lendemer 2005; Knudsen & Standley 2007), *S. plicata* (Magnusson 1935b; Knudsen & Kocourková 2009),

S. "*privigna*" (Magnusson 1935a, b; Harris & Ladd 2005; Knudsen & Standley 2007), *S. reebiae* K. Knudsen (Knudsen & Standley 2007; Knudsen et al. 2011), *S. regularis* Körb. (Knudsen 2007; Harris & Ladd 2005), *S. similis* H. Magn. (Magnusson 1935b; Harris & Ladd 2005; Knudsen & Standley 2007), and *S. sphaerospora* J. Steiner (Lendemer et al. 2009b). Three species are known only from lost type collections: *S. athroocarpa* H. Magn., *S. integra* B. de Lesd. ex H. Magn., and *S. magnussonii* B. de Lesd. (Magnusson 1935b; Knudsen & Standley 2007). We have not seen any taxa fitting the descriptions of these three species that could be used for neotypification.

We are currently researching the biodiversity and taxonomy of the lichenized, lichenicolous, and rock-inhabiting fungi of Joshua Tree National Park. In the park, *Sarcogyne plicata*, originally described from California (Magnusson 1935b), is sympatric with *S.* "*privigna.*" Knudsen & Standley (2007) previously treated *S. plicata* as a synonym of *S.* "*privigna*" because it was unclear how to delimit many specimens from *S.* "*privigna*" as then interpreted (see discussion below). Unpublished molecular work by Martin Westberg (Westberg, pers. comm.) supported *S. plicata* as distinct from *S.* "*privigna,*" and we accepted both species although we were still unclear how to differentiate the two (Knudsen & Kocourková 2009). In this paper we revise *Sarcogyne plicata*.

Materials & methods

Specimens were examined from COLO, FH, NY, UCR, and UPS. Specimens were studied in hand sections using standard microscopy. Structures were examined in water and KOH and measured in water. Amyloid reactions were tested with IKI. Hymenial height does not include the height of the subhymenium, a valuable secondary character in distinguishing some species. Thin-layer chromatography was performed by J.C. Lendemer on *Sarcogyne plicata*, Knudsen 1230 (NY) (Culberson & Kristinsson 1970). Images were captured using an Olympus DP20 digital camera with Microsuite Special Edition. The illustrations were prepared using Adobe Photoshop. All measurements are based on water mounts of hand cut sections unless otherwise indicated.

The species

Sarcogyne plicata H. Magn., Ann. Crypt. Exot. 7: 134 (1935). FIG. 1

TYPE: **U.S.A. CALIFORNIA.** SAN BERNARDINO CO., Upland. On plaster of wall. 17.11.1917, I.M. Johnston s.n. (Holotype, FH!).

DESCRIPTION — Thallus endolithic to chasmolithic, sometimes visible between granules of substrate or beneath apothecia as white cushion of plectenchyma. Vegetative hyphae mostly 1–2 µm in diam., cells mostly 4–5 µm long, thin-walled, I–, mixed with crystals of substrate, forming a gelatinized mass binding the substrate, continuous with the hypothecium and excipulum hyphae, not forming a distinct medulla. Algal layer usually scattered in substrate or not observable, algae chlorococcoid 10–15 µm in diameter.

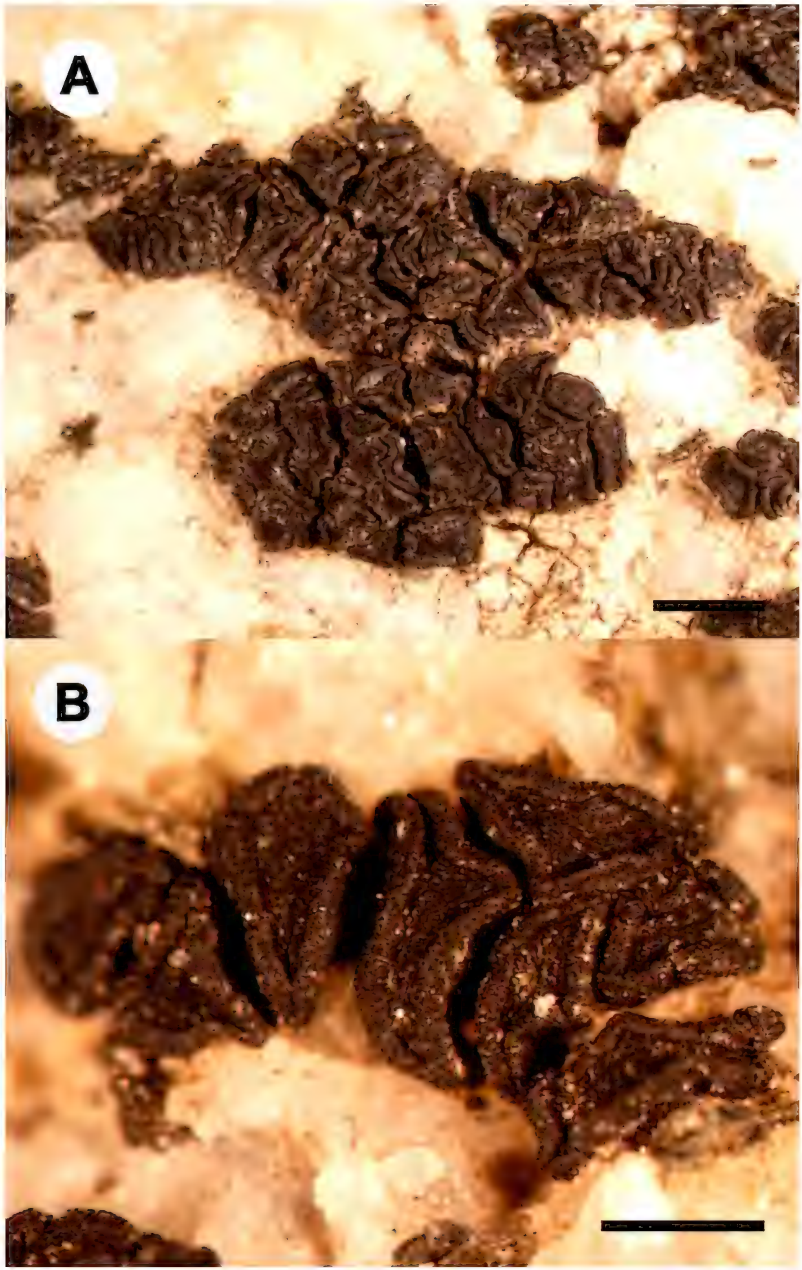

Fig. 1. *Sarcogyne plicata* (Knudsen 1230).
A. Apothecia. Scale = 1mm; B. Apothecia. Scale = 0,5 mm.

Apothecia usually contiguous and apparently not dividing vegetatively, dull black, epruinose, black when wetted, round, angular, to long and narrow (then vaguely resembling an *Opegrapha*), the disc black, visible or obscure, not turning red when wetted, often less than 0.5 µm in diameter or in length, up to 1 mm. Surface of disc without epihymenial accretions as in *Polysporina* species but rarely with an umbo, which is a remnant of the ontogeny of the apothecium. Excipulum distinct, melanized, up to 100 µm thick, outer layer dark (hyphae not visible), inner layer to 40 µm thick, golden yellow to red-brown, raising above the disc and even hiding it when dry, not melanized beneath the disc, sometimes swollen like labia, or in segments fused often at angles to each other through compression. Hymenium 100–140 µm tall, hyaline, I+ blue; epihymenium golden yellow, to 15 µm tall, thick, paraphyses septate, mostly 1.5–2.0 µm tall, with oil drops, with moderate branching, upper three cells sometimes becoming wider to 4 µm, apices usually expanded up to 4 µm, often in pigment cap. Asci 70–85 × (17–)20–25 µm, over 100 ascospores per ascus. Ascospores hyaline, simple, 3–5 × 1–2 µm, variable in size. Subhymenium golden yellow, to 50–80 thick, I+ blue. Hypothecium continuous with attaching hyphae, forming the base of apothecia, to 300 µm thick, without algal layer. Conidiomata not seen. No lichen substances found with TLC.

Selected specimens examined — **U.S.A. California.** Orange Co., Santa Ana Mountains, Fremont Canyon, on granite, 268 m, Sept. 22, 2008, K. Knudsen 10329 (UCR); on granite rocks eroded out of sandstone, 343 m, Dec. 6, 2007, K. Knudsen 9354 (UCR); 200 m, on conglomerate rock, Oct. 5, 3007, K. Knudsen 9004.2 (UCR); 956 m, Sept. 18, 2008, K. Knudsen 10310 (UCR); Riverside Co., Wildomar, Menifee Hills, on granite wall along seasonal stream, 558 m, Jan. 2, 2004, K. Knudsen 2124 (UCR); 635 m, granite drainages, Dec. 28, 2005, K. Knudsen 4837 (UCR); 543 m, crumbling granite along stream, Oct. 14, 2009, K. Knudsen 11782 (UCR); Joshua Tree National Park: Pleasant Valley, wash crossing Geology Tour Road, 1049 m, on flat granite rock, Dec. 3, 2010, K. Knudsen 12689 (UCR); Malapai Hill, south-facing base on granite rubble, 1165 m, Dec. 2, 201, K. Knudsen 12636.1 & 12636.2 (UCR); Squaw Tank, on dissected decaying quartz dike on monzogranite dome, 1092 m, Dec. 4, 2010, K. Knudsen 12748 (UCR); desert between Skull Rock & Jumbo Rocks, 1347 m, on monzogranite, Dec. 19, 2010, K. Knudsen 13150 (UCR); San Jacinto Mountains, along Forbes Ranch Road, on granite on pebble plain, 1651 m, March 26, 2006, K. Knudsen 5698 (UCR); San Bernardino Co., Granite Mountains, above Granite Cove, along wash, 1335 m, on granite slab, June 6, 2008, K. Knudsen 9687 (UCR); Joshua Tree National Park, Key's Ranch, 1267 m, on granite in washes, May 26, 2005, K. Knudsen 3041 w/ T. LaDoux (UCR); San Gabriel Mountains, San Antonio Creek, in wash behind San Antonio Dam, 761 m, on hard granite boulders in flood plain, June 4, 2004, K. Knudsen 1230 (ASU, MIN, NY, UCR); Santa Ana River Valley, base of San Bernardino Mountains, along Greenspot Road, on granite boulders in floodplain, 533 m, May 1, 2006, K. Knudsen 5936 w/ M. Knudsen (UCR); Woolly Star Preserve, 327 m, on granite boulder, Feb. 7, 2011, K. Knudsen et al. 13570 (UCR); Ventura Co., Santa Monica Mountains, Tri-Peaks near Backbone Trail, 829 m, on Conejo volcanics, May 13, 2007, K. Knudsen 8397 (UCR); Yerba Buena Canyon area, 409 m, March 22, 2006, K. Knudsen 5623 w/ D. Magney (UCR).

Ecology & distribution — *Sarcogyne plicata* is frequent in southern California, often locally abundant, usually occurring on granite in drainages, washes and flood plains, often a solitary pioneer in full sun, at elevations from 200 to 1651 meters. A few collections from the Santa Monica Mountains are on Conejo volcanics. No populations have been found on calcareous rock. It is currently considered endemic to California, occurring in the Mojave Desert (Granite Mountains and Joshua Tree National Park) and in the coastal mountains and foothills of southern California in the Peninsular Ranges (Menifee Hills, Santa Ana Mountains, San Jacinto Mountains) and in the Transverse Ranges (San Bernardino Mountains, San Gabriel Mountains, and Santa Monica Mountains).

Discussion — A typical *S. plicata* specimen (Fig. 1) is quite distinctive from *S.* "*privigna*" (Fig. 2), but many specimens can be quite similar. For instance, a *S. plicata* thallus with many round apothecia may resemble *S.* "*privigna,*" while a *S. "privigna*" thallus with many compressed apothecia may be identified as *S. plicata*. While *S.* "*privigna*" usually has discs that appear red even when dry, some thalli have mostly blackish apothecia. Fortunately *S.* "*privigna*"

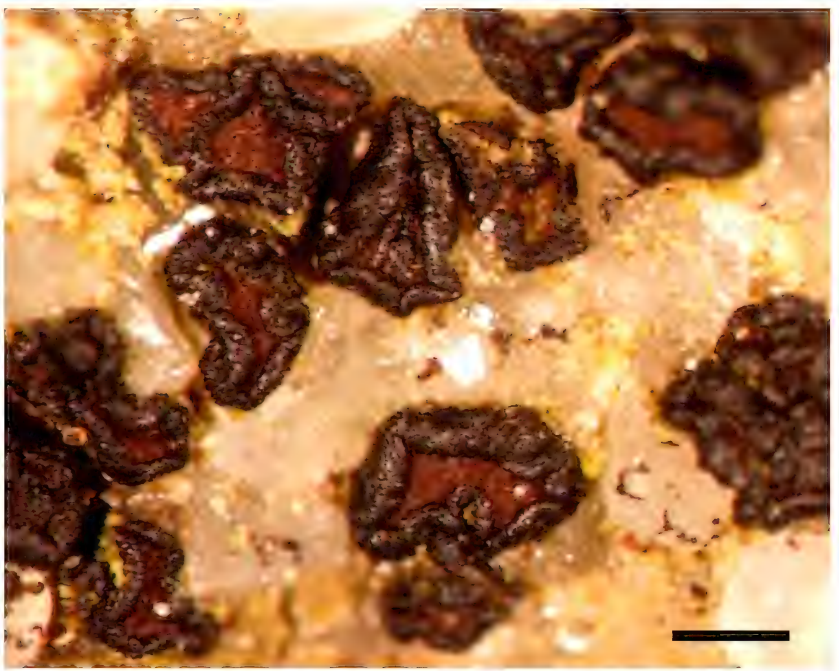

Fig. 2. *Sarcogyne 'privigna'* (Harris 42777).
Apothecia. Scale = 250 µm.

discs always turn red when dampened with water while *S. plicata* discs remain black. Otherwise *S. plicata* has a taller hymenium (100–140 µm) and deeper subhymenium (50–80 µm) than *S.* "*privigna*" (60–85 µm and 30–40 µm, respectively). One site where both species occurred was in Joshua Tree National Park at Key's Ranch. The site has extensive monzogranite boulders forming a maze of small alkaline washes, which commonly contain rock crevices and fissures rich in calcium deposited during seasonal flooding and subsequent evaporation. *Sarcogyne* "*privigna*" was common in these calcareous microhabitats. *Sarcogyne plicata* was frequent in the more acidic microhabitats found on the tops of monzogranite boulders that are not inundated during flooding and thus lack calcium deposits. In areas of the Mojave Desert like Cactus Flats (San Bernardino Mountains) or in the Clark Mountains, where limestone predominates, *S.* "*privigna*" was abundant and no *S. plicata* was collected.

The problem of *Sarcogyne "privigna"* auct., non (Ach.) A. Massal. FIG. 2

Lichenologists generally agree as to which taxon should be referred to *Sarcogyne* "*privigna*" (for instance: Magnusson 1935a, b; Clauzade & Roux 1985; Golubkova 1988; Knudsen & Standley 2007; Seppelt et al. 1998; Fletcher & Hawksworth 2009). However, the Acharius type of the basionym *Lecidea privigna* Ach. 1803 (and thus Massalongo's 1854 combination in *Sarcogyne*) represents *Polysporina simplex* (Taylor) Vězda (Magnusson 1935a). The Acharius types of the other possible basionym, *Lecanora milvina* var. *privigna* Ach. 1810, appear to represent *Polysporina simplex* and/or an *Acarospora.* For descriptions see Clauzade & Roux (1985), Magnusson (1935a, b), Knudsen & Standley (2007), and Fletcher & Hawksworth (2009). Thus the *S.* "*privigna*" now recognized by many authors does not have a validly published name (Magnusson 1935a; Knudsen & Standley 2007).

Sarcogyne plicata is restricted to a small area within the range of the more cosmopolitan *S.* "*privigna*". As noted above *S.* "*privigna,*" can generally be separated from the often similar to *S. plicata* based on its shorter hymenium and shallower subhymenium. The species also sometimes retains a vestige of apothecial development in the form of an umbo in the center of the disc that usually eventually disappears. Also as noted above, the easiest way to differentiate the two species is that *S.* "*privigna*" discs (usually red or red-orange when dry) are brighter when wet, while the black *S. plicata* discs remain black even when wet. The *S.* "*privigna*" pycnidia we observed were ca. 0.1–0.2 mm diam., while conidia were simple, hyaline, and 1.0–2.0 × 0.5–1.0 µm.

SELECTED SPECIMENS EXAMINED — **CZECH REPUBLIC.** BOHEMIA, Jeseníky, 500 m, May 9, 1961, A. Vězda, Lichenes Selecti Exsiccati 95 (COLO); **GREECE.** RETHIMNO, Mt. Psiloritis, on calcareous rock, 800 m, March 31, 1993, A. Nordin 3129 (UPS); SAMOS: west end of the island, 156 m, on limestone, D.J. Hill s.n. (UCR); **ITALY.** LOMBARDY,

Bormio, 1250 m, on stone fence, July 24, 1927, A.H. Magnusson 10675 (UPS); **NORWAY.** Troms, Storfjord Par., rocky slope, 60 m, on horizontal calcareous rock, Aug. 6, 2003, A. Nordin 5633 (UPS); **SLOVAKIA.** Bratislavský Svätý Jur, in vineyard, July 20, 1922, A.H. Magnusson 6825 (UPS); **SWEDEN.** Gotland, Fårö par., Gotska Sandön, on human bone, May 14, 1921, T. Vestergren (UPS); Lyckselle Lappmark, Tärna par., 520 m, Aug. 30, 1963, G.E. Du Rietz 675D (UPS); Södermanland, Östra Vingåker par., on calcareous rocks, Aug. 11, 1913, A.H. Magnusson s.n. (UPS) Västergötland, Lerdala pars., Sparresäter, on rocks in wood, July 12, 1934, A.H. Magnusson 14261 (UPS); Meddelplana par., Hällekis, on gneiss, July 12, 1942, A.H. Magnusson 18214 (UPS); **U.S.A. California.** Los Angeles Co., Santa Monica Mountains, Hennessey, south of Castro Crest, 445 m, on calcium carbonate accretions on sandstone, May 31, 2009, K. Knudsen 11169 (UCR); Orange Co., Santa Ana Mountains, Fremont Canyon, 587 m, on sandstone, Sept. 18, 2008, K. Knudsen 10303 (UCR); Riverside Co., Joshua Tree National Park, Hexie Mountains, Stirrup Tank, 1040 m, on granite, Dec. 26, 2010, K. Knudsen 13313 (UCR); base of Hexie Mountains at head of Fried Liver Wash, 983 m, rare on granite rocks along wash, Dec. 12, 2010, K. Knudsen 13013 (UCR); Ryan Mountain, 1568 m, on small granite rock in drainage, Dec. 6, 2010, K. Knudsen 12842 (UCR); Sheep's Pass, west-facing slope covered with rubble and pebbles, 1369 m, on decaying granite, Dec. 20, 2010, K. Knudsen 13218 (UCR); Upper Juniper Flats, on soft granite, 1497 m, Dec. 18, 2010, K. Knudsen 13107 (UCR); San Bernardino Co., Clark Mountains, 1535 m, on limestone, Oct. 11, 2009, K. Knudsen 11768 w/ N. Pietrasiak (UCR); Granite Mountains, canyon above Yucca Bajada camp, 1219 m, Oct. 20, 2007, K. Knudsen 9391 (UCR); Joshua Tree National Park, Key's Ranch, on monzogranite in alkaline wash, 1283 m, Apr. 1, 2005, K. Knudsen et al. 2622 (UCR); San Bernardino Mountains, Cactus Flats, 1905 m, common on calcareous rock, June 7, 2005, K. Knudsen 3284 (UCR); Bear Mountain, 2652 m, on dolomite, Aug. 25, 2005, K. Knudsen 1609 w/ C.L. Wagner (ASU, UCR); pebble plain along Polique Canyon Road, on granite rock, 2282 m, Aug. 25, 2011, Knudsen 13674 w/ S. Eliason (UCR); San Diego Co., Anza Borrego, Sentenac Canyon, 677 m, on granite in drainage, Dec. 23, 2005, K. Knudsen 4832 et al. (UCR); Laguna Mountains, The Point, 1825 m, on vertical granite walls, June 2, 2004, K. Knudsen 1223 (ASU, UCR); Santa Barbara Co., Santa Rosa Island, bottom of Dry Canyon, 96 m, on sandstone along seasonal stream, Oct. 24, 2008, K. Knudsen 10538 (UCR); **Georgia.** Putnam Co., Oconee National Forest, on sandstone, Sept. 16, 1996, W.R. Buck 30477 (NY); **Kansas.** Russell Co., west of Bunker Hill, 479 m, on sandstone, June 25, 2007, C.A. Morse 15459A (KANU, UCR); **Kentucky.** Harlan Co., Profile Rock, 763 m, on sandstone, Sept. 16, 1991, R.C. Harris 27175 (NY); **Maine.** Kennebec Co., Mud Pond, disturbed area under power lines, Sept. 19, 1987, on granite, R.C. Harris 20896 (NY). **Maryland.** Anne Arindel Co., On Wishing Rock, Raritan formation, Nov. 25, 1987, Clyde F. Reed (NY); Frederick Co., Sugar Loft Mountain, on rocks along Mt. Ephram Rd., Apr. 19, 1962, Clyde F. Reed (NY); **Missouri.** Carter Co., bluff on e-side of Current River, on top of granite boulder, R.C. Harris 25629 (NY); Madison Co., Castor River Shut-Ins Natural Area, on granite bedrock, Oct. 21, 2001, R.C. Harris 45075, 45069-A (NY). **New Mexico.** San Juan Co., Chaco Canyon National Monument, 1090 m, on sandstone, Aug. 6, 1979, T.H. Nash III, 16362 (ASU); **North Carolina.** Jackson Co., Cedar Cliff Mountains, Oct. 11, 1998, on schist and gneiss, R.C. Harris 42777 (NY); **Ohio.** Gallia Co., Wayne National Forest, Symes Creek Natural Area, 215 m, May 21, 2006, on sandstone, W.R. Buck 50330 (NY); **Pennslyvannia.** Blair Co., Tussey Mountain, on sandstone talus, Apr. 22, 2008, R.C. Harris 54291 (NY); Franklin Co., Michaux State Park, n-slope of Rocky mountain, c. 350 m, June 1, 2009, J.C. Lendemer 18182 (NY); Lycoming Co., Tioga State Forest,

Algerine Swamp Natural Area, c. 548 m, May 14, 2009, on rock, J.C. Lendemer 16992 (NY); **Vermont.** Lamoille Co., Smuggler's Notch, rocks at base of a cliff, Sept. 21, 1985, R.C. Harris 18222 (NY); **West Virginia.** Grant Co., Shroud Ridge, on sandstone, 425 m, W.R. Buck 38252 (NY).

Ecology & distribution — *Sarcogyne "privigna"* occurs on calcareous rock as well as non-calcareous rock such as granite, gneiss, sandstone and volcanic rock in drainages, washes, and flood plains potentially rich in calcium carbonate deposited in the fissures of rocks during inundation and subsequent evaporation. The species is widespread and found in Africa, Asia, Europe, North America, Antarctica, and Australia (Fletcher & Hawksworth 2009; Golubkova 1988; Seppelt et al. 1998).

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Homolaphlyctis polyrhiza gen. et sp. nov., a species in the *Rhizophydiales* (*Chytridiomycetes*) with multiple rhizoidal axes

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Abstract — An undescribed cellulosic chytrid with multiple rhizoidal axes, JEL142, has grouped in molecular hypotheses with *Batrachochytrium dendrobatidis*, the chytrid pathogen of amphibians, and thus is of interest for genetic and physiological comparisons. To describe this member of the *Rhizophydiales*, we examined its zoospore ultrastructure and developmental morphology. Based on a reanalysis of rDNA data plus ultrastructural and morphological characters, we name this fungus *Homolaphlyctis polyrhiza* gen. et sp. nov.

Key words — *Chytridiomycota*, phylogeny, *Rhizophlyctis*, TEM

Introduction

A chytrid referred to by its isolate number, JEL142, has been in phylogenies since the first molecular hypotheses of the *Chytridiomycota* (James et al. 2000, 2006; Letcher et al. 2008b) but has not been identified to species or received a formal name. This isolate is of interest because it groups as sister to *Batrachochytrium dendrobatidis* Longcore et al. (Longcore et al. 1999), the pathogen of amphibians, and because it is one of the few members of the *Rhizophydiales* Letcher (Letcher et al. 2006) that have thalli with multiple rhizoidal axes. Although the phylogenetic distance based on branch lengths in phylograms is large and support values for the relationship have been low (James et al. 2006; Letcher et al. 2008b), JEL142 seems to be the closest relative to *B. dendrobatidis* now in culture. In an effort to identify genes that enable

B. dendrobatidis to be pathogenic, the genome of JEL142 has been sequenced for comparison (Joneson et al. 2011). In this paper, we reanalyze the phylogenetic placement of JEL142 and provide a description of the ultrastructure of its zoospore and its developmental morphology. These analyses place isolate JEL142 in the *Rhizophydiales,* but outside of described families or genera. Therefore we describe a new genus and species to accommodate isolate JEL142.

Materials & methods

We first observed this chytrid on onionskin bait (outer, dry bulb scales of white onions) placed in a gross culture containing the aquatic plant *Eriocaulon aquaticum*, associated debris and lake water in a deep Petri dish. The collection site was Mud Pond, a small (1.7 ha), acidic (pH 4.6), oligotrophic and fishless lake in Hancock County, Maine (Davis et al. 1994, Rhodes & Davis 1995). With standard methods (Barr 1986) we isolated JEL142 into pure culture on modified PmTG nutrient agar (Longcore 1992). Since initial isolation in 1994 this chytrid has grown on nutrient agar slants with transfers at three-month intervals and storage at ~4 °C after growth. We collected zoospores from growth on 20 mPmTG plates and fixed them for TEM with s-collidine buffered glutaraldehyde followed by osmium tetroxide. Zoospores were rinsed, added to agar and en bloc stained with uranyl acetate before embedment in epon araldite plastic (Barr 1981, Longcore 1992). Sections were examined at 60 kV. We photographed developmental morphology of thalli grown on mPmTG nutrient agar with a Spot RT digital camera. Phylogenetic analysis of *Rhizophydiales* was conducted using the initial alignments of 18S, 28S, and 5.8S rRNA subunits produced by James et al. (2006). Additional sequences were added from Letcher et al. (2004, 2008b), and the alignment manually adjusted in MacClade 4.08 (Maddison & Maddison 1992). The phylogeny was estimated using maximum likelihood in RAxML-7.0.4 (Stamatakis 2006) with 100 heuristic searches beginning from random trees. Support for nodes was established by bootstrapping in RAxML (1000 pseudo-replicates) and Bayesian posterior probabilities in MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001).

Results

The phylogenetic analysis of *Rhizophydiales* based on combined 18S, 28S, and 5.8S subunits provides strong support, 77 bootstrap percentage and 0.99 posterior probability, for a sister relationship between *H. polyrhiza* and *B. dendrobatidis* (Fig. 1). These two species group with a strain identified as *Entophlyctis helioformis* (JEL326), and this clade is sister to the other members of the *Rhizophydiales*. As seen in previous studies, the phylogenetic distance between *H. polyrhiza* and *B. dendrobatidis* is rather large as suggested by branch lengths, and the percentage difference between rRNA sequences of the two species is ~10%.

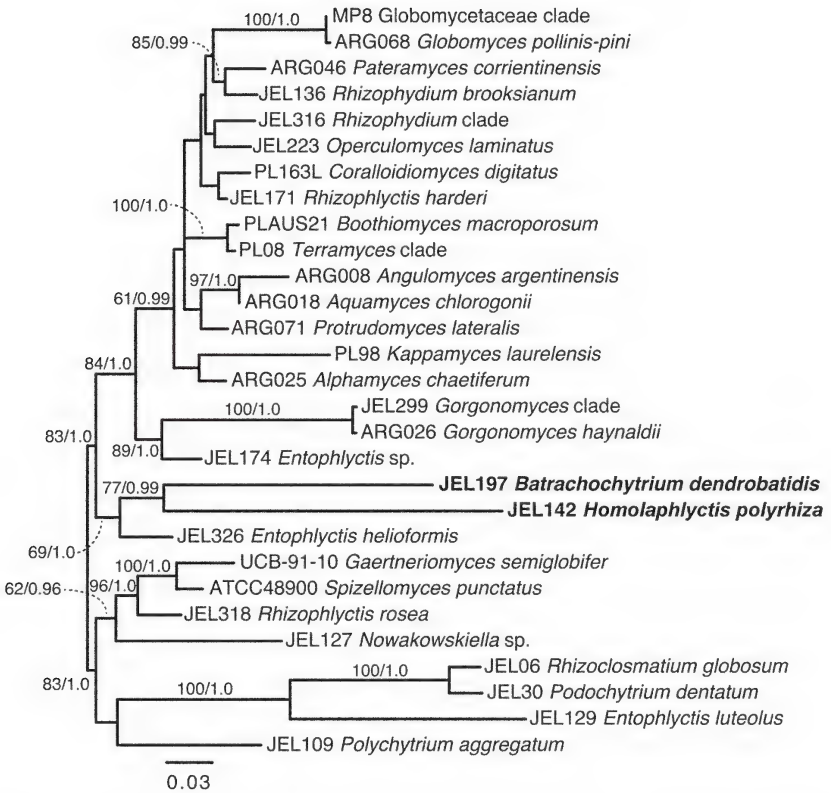

FIGURE 1. Phylogeny of *Rhizophydiales* showing sister relationship between *Homolaphlyctis polyrhiza* and *Batrachochytrium dendrobatidis*. The phylogeny was based on a combined analysis of 18S, 28S and 5.8S ribosomal RNA subunits and was estimated using maximum likelihood under a GTR+I+Γ model of evolution with RAxML-7.0.4 (Stamatakis 2006). Values at nodes indicate support as estimated by maximum likelihood from 1,000 bootstrap pseudo-replicates followed by Bayesian posterior probabilities. Only nodes receiving >60% bootstrap support are labeled. Further strain details and GenBank accession numbers can be found in James et al. (2006) and Letcher et al. (2004, 2008b).

Taxonomy

Homolaphlyctis Longcore, Letcher & T.Y. James, **gen. nov.**

MYCOBANK MB561579

Zoosporarum ultrastructura Rhizophydialium characteristica, constans ex microtubulis separatis duo vel tres e kinetosomate ad rumposoma in globulo solitario extensorum; microcorpus in latere interiore globuli lipoidei. Calcar kinetosomale et zona vesiculata

circum kinetosoma absunt. Mitochondrion lobatum. Vesiculae corde denso per cytoplasma dispersae.

Type species: *Homolaphlyctis. polyrhiza* Longcore, Letcher & T.Y. James

Etymology: Named for R.L. Homola in whose laboratory this work began, and A.D. Homola, who helped collect the sample.

Zoospore ultrastructure typical for members of the *Rhizophydiales*, consisting of stack of 2–3 separate microtubules leading from the kinetosome to a rumposome on a single lipid globule; microbody on inner side of lipid. Lacking kinetosome-associated spur and vesiculated area around kinetosome. Mitochondrion lobed. Dense-cored vesicles throughout cytoplasm.

Homolaphlyctis polyrhiza Longcore, Letcher & T.Y. James, **sp. nov.** Figs. 2, 3

MycoBank MB561580

Thallus axibus rhizoidalibus tres vel pluris extense dispersis. Papillae evacuationis tubulosae numerosae, breves usque ad longae.

Type: Fig. 2a–h **(holotype)** photographed from pure culture JEL142 isolated from cellulosic substrate placed with a collection of *Eriocaulon aquaticum* collected from Mud Pond, (44°38′00.26″N 68°05′15.81″W) Hancock County, Maine on 15 April 1994. [GenBank DNA sequences: AH009051 (SSU rDNA); EF634247 (LSU rDNA); EF634249 (ITS1-5.8S-ITS2).]

Etymology: The epithet refers to the multiple rhizoidal axes.

Thallus with three or more widely distributed rhizoidal axes. Multiple, short to long tubular discharge papillae.

Expanded Description — The generation time for *H. polyrhiza* on mPmTG nutrient agar is 2–3 days at 23 °C. In motion, zoospores are spherical (Fig. 2h, arrowhead) and contain a single lipid globule near the base of the flagellum (Fig. 2a). They encyst and within 24 h form a thallus with multiple rhizoidal axes (Fig. 2b). At maturity rhizoids can extend to more than twice the diameter of the zoosporangium (Fig. 2c); distally rhizoids are fine (<0.5 μm) and isodiametric, but near the thallus they are slightly swollen (Fig. 2d). Zoosporangia form discharge papillae (Figs. 2e, f) within 52 hr, with the number of papillae increasing with size of the zoosporangia. Papillae may be barely raised (Fig. 2f), short tubes (Fig. 2e) or, in crowded conditions, long tubes (Fig. 2g). Zoospores are elongate when discharged (arrows, Fig. 2h) but soon become spherical (dia 3.5–4.5 μm with flagellum up to 28 μm).

Ultrastructural characters — The ultrastructure (Figs 3a–e) of the *H. polyrhiza* zoospore is nearly identical to Barr's illustration of a typical *Rhizophydium* zoospore (Barr & Hadland-Hartmann 1978). A membrane-bound aggregation of ribosomes is in the center of the zoospore, outside of which are the nucleus, a single lobed mitochondrion, and a single lipid globule (Figs. 3a, b). A lobe of a mitochondrion is often anterior to the kinetosome

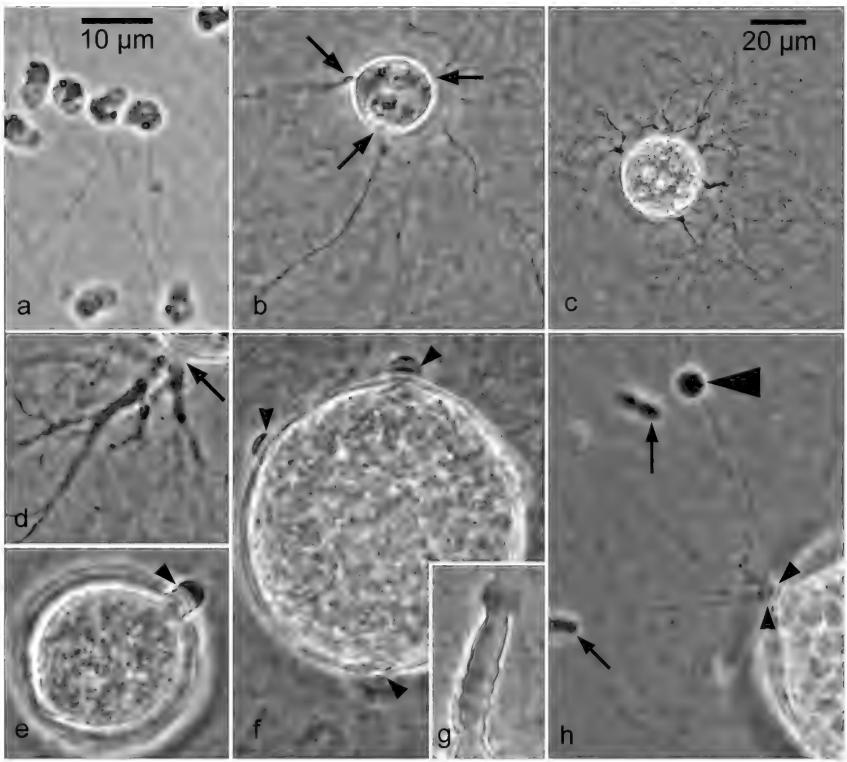

FIGURE 2. Developmental morphology of *Homolaphlyctis polyrhiza* in pure culture on mPmTG nutrient agar. a. Zoospores with single lipid globule, aggregated ribosomes and single posteriorly directed flagellum. b. Young thallus with multiple rhizoidal axes (arrows). c. Nearly mature thallus showing the extent of rhizoids. d. Rhizoidal bases (arrow) are enlarged. e. Papilla (arrowhead) on nearly mature zoosporangium. f. Short, evenly spaced discharge papillae (arrowheads). g. Long discharge papilla, nearly ready to release zoospores. Note breakdown of apical, hyaline papillar material. h. Zoospores (arrows) elongate during discharge through papillar opening (arrowheads) but become spherical shortly after release (large arrowhead). Scale bar in a, for all figures except c.

(FIG. 3a). The microbody is appressed to the side of the lipid globule toward the center of the zoospore (FIG. 3b), and a rumposome (= fenestrated cisterna of Letcher et al. 2004) is appressed to the lipid near the surface of the cell (FIG. 3b). The non-flagellated centriole is parallel to the kinetosome and joined to it by a bridge of partly overlapping fibers centrally located between the two structures (FIGS. 3c, d; arrow); the fibrils form a wide (>0.075 µm) zone of convergence. The non-flagellated centriole is shorter than its width (FIGS. 3c, e). A microtubular root, composed of separate, parallel microtubules, extends from the side of the kinetosome to the rumposome (FIG. 3e). A terminal plate is in the lumen of

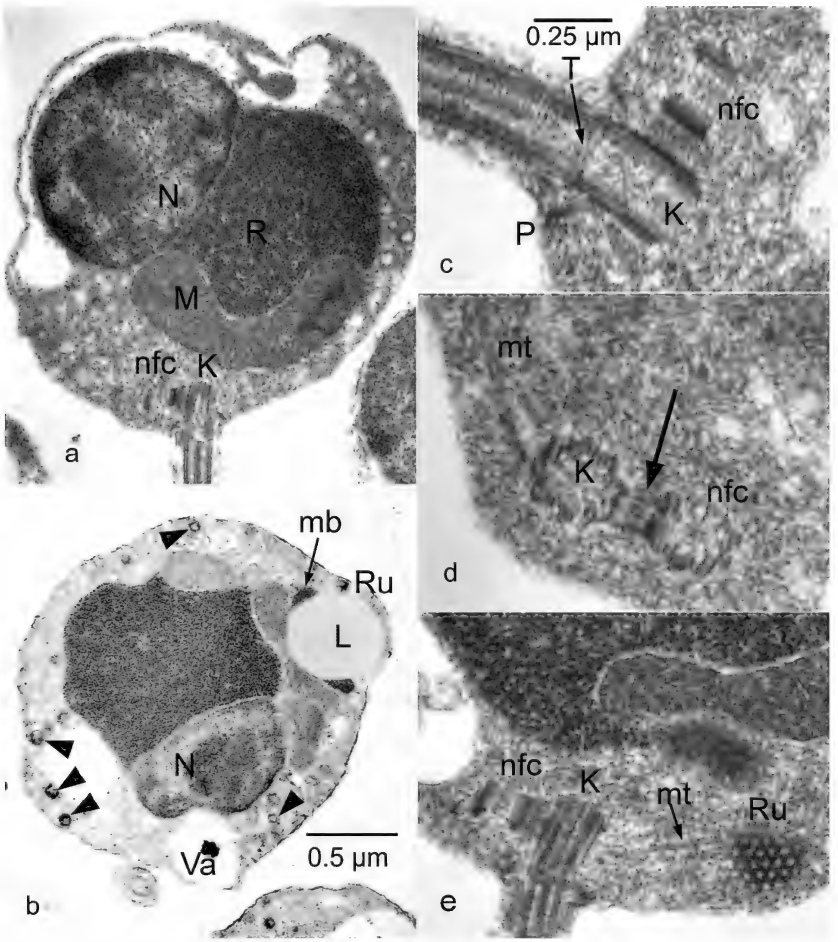

FIGURE 3. Ultrastructural features of *Homolaphlyctis polyrhiza* zoospores. a. Longitudinal section of zoospore with nucleus (N); aggregated ribosomes (R); lobed mitochondrion (M); kinetosome (K); non-flagellated centriole (nfc). b. Transverse section with lipid globule (L); microbody (mb); rumposome (Ru); vacuole (Va); and vesicles (arrowheads). c. Longitudinal section of kinetosomal region with kinetosomal props (P) and terminal plate (T). d. Cross section of kinetosome and non-flagellated centriole with fibrillar connection and wide zone of convergence (arrow) between the two structures. Microtubule root (mt) extends from the kinetosome. e. Longitudinal section through the kinetosome and non-flagellated centriole; microtubules extend from the kinetosome to the rumposome, shown in face view. Scale bar in b also for a; scale bar in c also for d–e.

the axoneme (FIG. 3c). Typical for members of the *Rhizophydiales* (Letcher et al. 2006), a flagellar plug is absent from the transition region of the flagellum. In the peripheral cytoplasm outside the central core are numerous, large

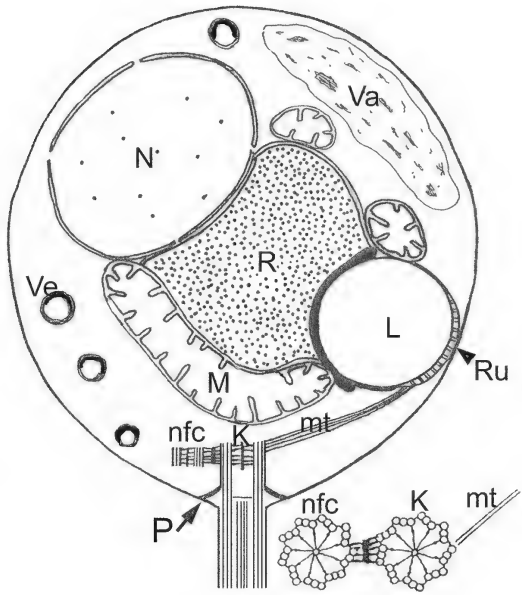

FIGURE 4. Summary diagram of zoospore ultrastructure of *Homolaphlyctis polyrhiza*. Kinetosome (K); lipid globule (L); mitochondrion (M); microbody (mb); microtubule root (mt); nucleus (N); nonflagellated centriole (nfc); prop (P); vacuole (Va); vesicle (Ve).

(~200 nm diameter) cored vesicles (FIG. 3b; arrowheads); a large vacuole is often present. Ultrastructural features are summarized in FIG. 4.

COMMENTS — We found this saprobic organism only once, from an oligotrophic, acidic lake. Based on descriptions of genera of the *Chytridiales* sensu Sparrow (1960), it would have been placed in the genus *Rhizophlyctis* A. Fisch. because it develops endogenously and has multiple rhizoidal axes. Molecular and TEM evidence have altered our concept of the importance of morphological characters (e.g., James et al. 2000, 2006; Mozley-Standridge et al. 2009) and chytrids with endogenous development and multiple rhizoidal axes occur not only in the *Rhizophlyctidales* (Letcher et al. 2008a) but also other orders of the *Chytridiomycetes*, now including the *Rhizophydiales*.

Acknowledgments

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Eutypella phaeospora, a new species on *Chenopodiaceae*

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Abstract — The authors describe a new species of *Eutypella* (*Diatrypaceae*, *Xylariales*) based on several collections on *Suaeda vera* in France and one on *Salsola vermiculata* in Spain. The *Libertella*-like asexual state was obtained in culture.

Key words — *Ascomycota*, taxonomy, salt marsh, disjunct distribution, semi-arid land

Introduction

An ascomycete referable to *Eutypella* (Nitschke) Sacc. was repeatedly collected on *Suaeda vera* J.F. Gmel. (*Chenopodiaceae*), a woody shrub occurring in salt marshes in Ile de Ré (France), and once on *Salsola vermiculata* L. (*Chenopodiaceae*) in a semi-arid environment in northern Spain. Salt marshes are known to be peculiar ecosystems in which halophytes associate with both terrestrial and marine mycota (Barata 2002). Previous investigations have been carried out mainly on monocots such as *Juncus roemerianus* (Kohlmeyer et al. 1995), *Spartina* sp. (Gessner & Kohlmeyer 1976), *S. alterniflora* (Gessner 1977), and *S. maritima* (Barata 2002), but little attention has been paid to woody shrubs.

Based on extensive collecting of ascomycetes in Ile de Ré, this *Eutypella* appears to be somewhat common in salt marshes but was never encountered on the island out of this biotope. However, its occurrence on *S. vermiculata* in a very different environment in Spain suggests a stronger relationship to *Chenopodiaceae* than to a specific ecological niche as first assumed.

As it is also remarkable in possessing ellipsoid brown ascospores, we decided to describe it as new. A typically diatrypaceous anamorph obtained in culture confirmed its placement in *Diatrypaceae* and a living culture was deposited in CBS, allowing further phylogenetic investigations in the future.

Materials & methods

Macro and microscopic observations were carried out following Rappaz's (1987) methodology, especially by testing the reaction of subapical ring to Melzer's reagent after pre-treatment in 3% KOH and by making measurements of asci and ascospores in heated lactic cotton blue. Micrographs of asci were taken in Lugol's solution (IKI), those of conidiogenous structures and conidia were taken in dilute Waterman blue ink and water. Cultures were made from single ascospores which were isolated on malt extract agar (Difco™ Malt Extract Agar) according to Rossman's (1999) method. The material collected, along with duplicates of holotype, is kept in the the authors' private herbaria (JF and CLL numbers). The holotype is deposited in LIP herbarium (Lille) and cultures at CBS.

Taxonomy

Eutypella phaeospora J. Fourn. & Lechat, **sp. nov.** PLATE 1

MYCOBANK 561649

Ab alteribus speciebus generis Eutypella *differt combinatione crescentiae in* Chenopodiaceae, *ascosporarum ellipsoidearum et brunneorum et apiculi annuli leviter amyloidei*.

TYPE: France, Charente Maritime: Ile de Ré, Loix, Le Feneau, edge of a salt marsh, on dead or dying stems of *Suaeda vera*, 16 Apr. 2008, leg. MH, CLL & JF (**Holotype**, LIP JF08078; ex-type culture, CBS129148).

ETYMOLOGY: The epithet is derived from the unusual brown colour of ascospores.

STROMATA corticolous or lignicolous, pustulate, pustules 0.8–1.8 mm diam, comprising 2–8 perithecia, irregularly scattered, separate to sometimes confluent; in bark, stromata irregularly rounded, hardly raising the periderm, under a conspicuous dorsal black line which is absent when the periderm is tightly adherent, cortical tissue hardly altered, without differentiated entostroma; in wood, stromata fully immersed to slightly raising wood surface, elongated in the grain of wood, substrate not altered but surface more or less blackened. OSTIOLES emerging collectively as small bunches 0.5–1 mm diam, shiny black, rectangular with cruciform apices and deeply sulcate sides, (80–) 150–840 µm high, 210–290 µm diam. PERITHECIA subglobose, 380–420 µm diam, in contact, with short to long convergent necks. ASCI unitunicate, thin-walled, long-pedicellate, the spore-bearing parts cylindrical to fusiform

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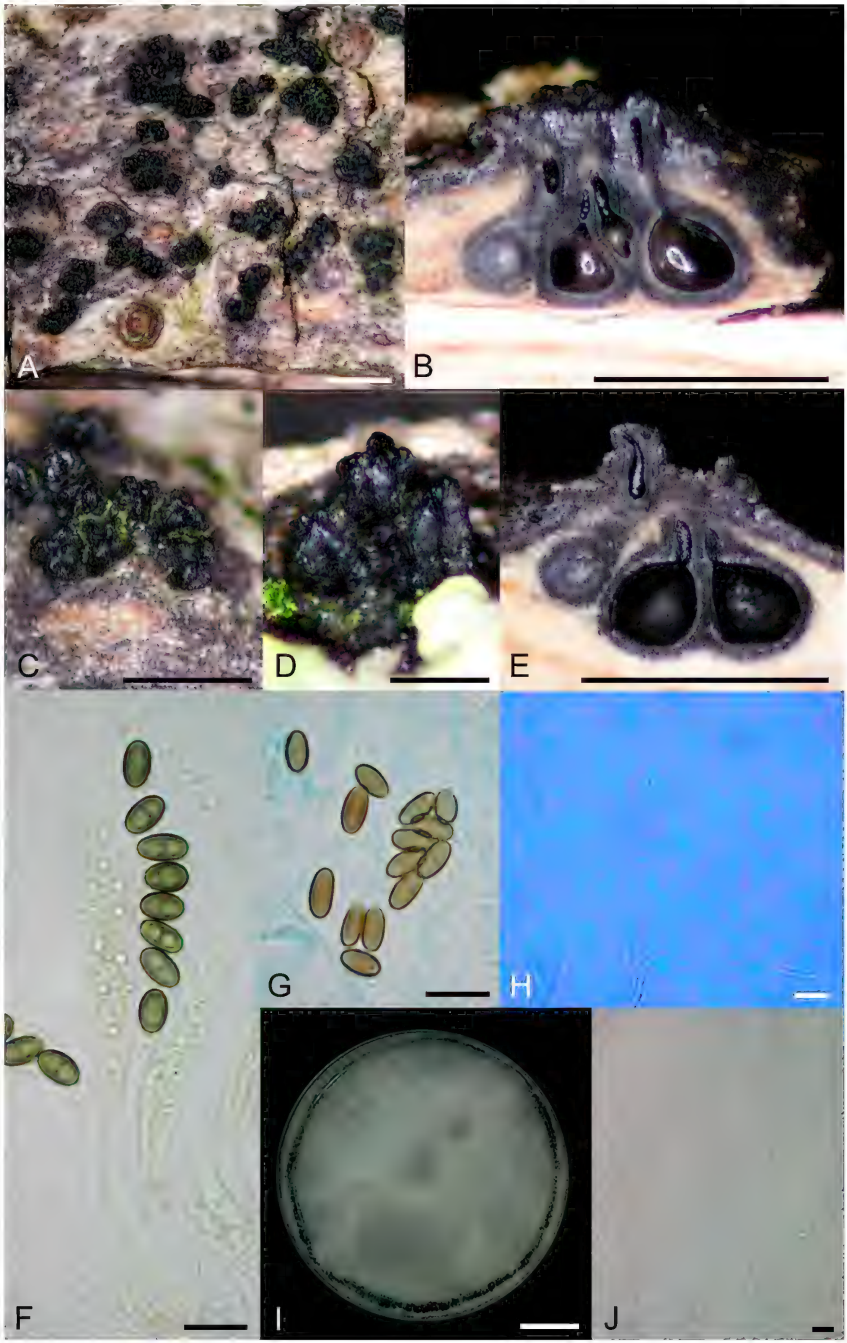
PLATE 1. *Eutypella phaeospora* (holotype). A: Surface view of host bark showing bunches of ostioles erumpent through the periderm. B: Cross section through a stroma in bark showing convergent ostiolar necks. C–D: close-ups showing the stout cruciform ostioles. E: Cross section through a stroma in wood showing convergent ostiolar necks. F: Mature and immature asci mounted in Lugol's solution showing the very faint reaction of apical ring. G: Ascospores mounted in lactic cotton blue. H: Conidiophores, conidiogenous cells and conidia of the *Libertella*-like anamorph mounted in dilute Waterman blue ink. I: Culture in Petri dish. J: Conidia in water.

Scale bars: A = 5 mm; B–C,E = 1 mm; D = 0.5 mm; F–H,J = 10 µm; I = 1 cm.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with a truncate apex, 35–40 µm long × 7.5–8.5 µm broad with a minute, very inconspicuous subapical ring bluing in Lugol's solution or in Melzer's reagent after pre-treatment with 3% KOH, the stipes 30–35 µm long, filiform, very fragile. Paraphyses copious, persistent, hyphae-like, hyaline, septate, 4–5 µm at base, tapering above asci. Ascospores 6–7.5 × 3.4–4 µm, oblong to ellipsoid-equilateral with broadly rounded ends, brown, one-celled, smooth, obliquely uniseriate to irregularly biseriate in the ascus.

Additional specimens examined: **FRANCE, Charente Maritime**: Ars en Ré, edge of a salt marsh, on dead or dying stems of *Suaeda vera*, 07 May 2007, leg. C. Lechat, CLL7129. **SPAIN, Navarra**: Arguedas, 80 km south of Pamplona, on roadside in the semi-desert of Bardenas Reales, 310 alt., on a dead corticated stem of *Salsola vermiculata*, 26 Sep. 2010, leg. Jean-Paul Priou, CLL10033.

Anamorph in culture: After two months on MEA, colony wrinkled, 2.5–3 cm diam., 0.3–0.5 cm high, producing a *Libertella*-like anamorph, white at first, then covered by greyish-white hyphal elements, white at margin, diffusing a brown to blackish coloration in the medium; reverse side greyish-yellow in the center, blackish at margin. Conidiophores branched, conidiogenous cells cylindrical, producing holoblastic conidia 16–23(–26) × 1.1–1.3 µm, hyaline, white en masse, cylindrical, tapered at ends, irregularly curved, e.g. curved on upper third of length or regularly and strongly curved, produced in whitish clusters on the median part of the colony.

Discussion

This ascomycete meets all the key features of the family *Diatrypaceae* Nitschke in macromorphology, ascal morphology, and *Libertella*-like anamorph. It only deviates in possessing ascospores that are ellipsoid and brown instead of allantoid and yellowish as encountered in typical taxa. However, several species in *Anthostoma* Nitschke, *Cryptosphaeria* Ces. & De Not., *Diatrype* Fr. and *Eutypella* are known to have brown ascospores (Rappaz 1987, 1992), and several of them —*A. decipiens* (DC.) Nitschke, *C. subcutanea* (Wahlenb.) Rappaz, *D. whitmanensis* J. D. Rogers & Glawe, *E. grandis* (Nitschke) Sacc., *E. corynostomoides* (Rehm) Rappaz— produce such ellipsoid ascospores along with broadly allantoid ones (Rappaz 1987).

The new taxon appears best accommodated in *Eutypella* as defined by Rappaz (1987) based on its pustulate stromata with stout, converging ostiolar necks. Although the recent ITS sequence analysis of *Diatrypaceae* by Acero et al. (2004) showed that *Eutypella* as currently conceived is most likely polyphyletic, those authors did not propose any formal taxonomic changes. We therefore refer the present fungus to *Eutypella* and await further molecular studies that might shed some light on its affinities within *Diatrypaceae*. A case was made to reconsider its placement following Carmarán et al. (2006), who reinstated *Peroneutypa* Berl. for taxa differing from *Eutypella* in ascal morphology.

Although we did not use fluorescence microscopy to study the morphology of asci in *E. phaeospora*, the ascal shape and subapical ring clearly point towards type I as defined by the authors and therefore suggest a closer affinity with *Eutypella* as currently accepted (Rappaz 1987).

Eutypella phaeospora can be readily distinguished from other *Eutypella* species through its consistently ellipsoid brown ascospores and occurrence on members of *Chenopodiaceae*. The usually uniseriate ascospore arrangement in the ascus correlated with the cylindrical shape of its spore-bearing part is likewise a distinctive feature contrasting with the spindle-shaped ascus most often encountered in *Diatrypaceae*. The fungus seems to be a common saprobe occurring on *Suaeda vera* in France and on *Salsola vermiculata* in Spain.

Eutypella kochiana Rehm and *E. alsophila* (Durieu & Mont.) Berl. (which occur on *Chenopodiaceae* in marine environments) are worth notice here, but their asci have a clearly amyloid subapical ring and their ascospores markedly differ in being yellowish and allantoid. *Eutypella kochiana* var. *salsolae* Urries, collected on *Salsola vermiculata* in Spain, resembles *E. phaeospora* in slightly larger ascospores (4.8–8 × 1.8–2.2 µm —vs. 4.8–6 × 1.5–1.8 µm in *E. kochiana*) that are sometimes oblong to broadly ellipsoid (Rappaz 1987). However that variety still differs in having asci with a clearly amyloid subapical ring and narrower, yellowish ascospores. The above collection of *E. kochiana* var. *salsolae* does suggest that at least two different *Eutypella* species do occur on *Salsola vermiculata*, whereas *E. phaeospora* is the only *Eutypella* species known thus far to occur on *Suaeda vera*.

Based on many occurrences of *E. phaeospora* on *S. vera* in salt marshes in Ile de Ré and absence inland, it was first assumed that the new species was restricted to coastal environments. However, the material collected in Spain from a very different environment —a semi-arid area far inland— points towards a disjunct distribution not correlated with salt marshes. The fact that *Salsola vermiculata* is an invasive shrub that can thrive in both coastal wetlands and inland semi-arid areas may explain this apparent discrepancy. Therefore, it could be assumed that *Eutypella phaeospora* exhibits a strong preference for *Chenopodiaceae* species regardless of ecological niche. Occurrence of *E. phaeospora* on *S. vermiculata* in salt marshes is predictable but should be confirmed by further sampling in coastal areas. Whether *E. phaeospora* occurs on other members of *Chenopodiaceae* or possibly on other shrubs is likewise still unknown, and it is hoped the morphological characterization given above will help elucidate its apparently complex ecology.

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New combinations in *Lactifluus*. 1. *L.* subgenera *Edules*, *Lactariopsis*, and *Russulopsis*

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Abstract — In this first of a series of three papers, new combinations in the genus *Lactifluus* are proposed. This paper treats the subgenera *Edules*, *Lactariopsis,* and *Russulopsis* (all proposed here as new combinations in *Lactifluus*). In *Lactifluus* subg. *Edules*, eight combinations at species level are proposed. In *Lactifluus* subg. *Lactariopsis*, the following three new combinations are proposed at sectional level: *Lactifluus* sect. *Lactariopsis* with seven newly combined species, *L.* sect. *Chamaeleontini* with eight newly combined species, and *L.* sect. *Albati* with four newly combined species plus two species previously combined in *Lactifluus*. Finally, in *L.* subg. *Russulopsis*, eight new combinations at species level are proposed.

Key words — milkcaps, nomenclature

Introduction

Multigene-based phylogenies of the *Russulaceae* show that *Lactarius* and *Russula,* described by Persoon in 1797 and 1796 respectively, are not supported as two monophyletic clades (Buyck et al. 2008). *Russula* appears to be monophyletic only when a small group of species (*R.* subsect. *Ochricompactae*) is removed. This small group of species, which forms a clade where *Lactarius* and *Russula* are mixed, was described recently as the new genus *Multifurca* Buyck & V. Hofst. (Buyck et al. 2008).

Apart from the representatives that belong to *Multifurca*, *Lactarius* divides into two clades, a larger and a smaller one, and splitting the genus seems a better solution than lumping everything in a giant genus *Russula.*

In a proposal to conserve *Lactarius* with a conserved type (Buyck et al. 2010), arguments were given to justify the conservation of *Lactarius torminosus*

(Schaeff.: Fr.) Pers. as the type of *Lactarius*. This has the advantage of retaining the name *Lactarius* for the larger clade, so that only the smaller clade (containing 20% of the species) would require new combinations. Since most of the species in the smaller clade are tropical, almost all temperate milkcaps would remain in *Lactarius*.

The subgenera *Lactarius* subg. *Piperites*, *Lactarius* subg. *Russularia*, and *Lactarius* subg. *Plinthogalus* would constitute the larger genus *Lactarius* sensu novo, while the former subgenera *Lactarius* subg. *Lactarius*, *Lactarius* subg. *Lactariopsis*, *Lactarius* subg. *Russulopsis*, *Lactarius* subg. *Lactifluus*, and *Lactarius* subg. *Gerardii*, and the former section *Lactarius* sect. *Edules* would constitute the genus *Lactifluus* (Pers.) Roussel.

The proposal (Buyck et al. 2010) was supported by the Nomenclatural Committee for Fungi (Norvell 2011) and has been approved by the General Committee and accepted by the 2011 International Botanical Congress (Barrie 2011, McNeill et al. 2011). This first paper proposes new combinations in *Lactifluus*, recombinations of species from the subgenera *Lactariopsis* and *Russulopsis,* and the section *Edules.*

Taxonomy

Lactifluus subg. *Edules*

Molecular phylogenies show that *Lactarius* sect. *Edules* is a well-supported, monophyletic clade (Buyck et al. 2008). It is preferable to treat it at the subgenus rank. Currently, this group consists of eight species, all endemic for tropical Africa.

Lactifluus subg. ***Edules*** (Verbeken) Verbeken, **comb. nov.**

MycoBank MB 563158

≡ *Lactarius* section *Edules* Verbeken, Belg. J. Bot. 132: 176. 2000 ("1999").

Type species: *Lactarius edulis* Verbeken & Buyck

Lactifluus aureifolius (Verbeken) Verbeken, **comb. nov.**

MycoBank MB 563150

≡ *Lactarius aureifolius* Verbeken, Bull. Jard. Bot. Belg. 65: 198. 1996.

Lactifluus densifolius (Verbeken & Karhula) Verbeken, **comb. nov.**

MycoBank MB 563151

≡ *Lactarius densifolius* Verbeken & Karhula, Bull. Jard. Bot. Belg. 65: 200. 1996.

Lactifluus edulis (Verbeken & Buyck) Buyck, **comb. nov.**

MycoBank MB 563152

≡ *Lactarius edulis* Verbeken & Buyck, Champ. Comest. Ouest Burundi: 103. 1994.

Lactifluus inversus (Gooss.-Font. & R. Heim) Verbeken, **comb. nov.**

MycoBank MB 563153

≡ *Lactarius inversus* Gooss.-Font. & R. Heim, Bull. Jard. Bot. État 25: 78. 1955.

Lactifluus latifolius (Gooss.-Font. & R. Heim) Verbeken, **comb. nov.**
MycoBank MB 563154
≡ *Lactarius latifolius* Gooss.-Font. & R. Heim, Bull. Jard. Bot. État 25: 82. 1955.

Lactifluus nodosicystidiosus (Verbeken & Buyck) Buyck, **comb. nov.**
MycoBank MB 563155
≡ *Lactarius nodosicystidiosus* Verbeken & Buyck, Mycol. Res. 111: 794. 2007.

Lactifluus phlebophyllus (R. Heim) Buyck, **comb. nov.**
MycoBank MB 563156
≡ *Lactarius phlebophyllus* R. Heim, Candollea 7: 378. 1938.

Lactifluus roseolus (Verbeken) Verbeken, **comb. nov.**
MycoBank MB 563157
≡ *Lactarius roseolus* Verbeken, Bull. Jard. Bot. Belg. 65: 207. 1996.

Lactifluus subg. *Lactariopsis*

Three sections are recognized in this subgenus. One, *L.* sect. *Lactariopsis*, is characterized by species with a distinct veil resulting from pseudoangiocarpic development (Heim 1937) and is known only in tropical Africa and South America. *Lactifluus* sect. *Chamaeleontini* also has its major distribution in tropical Africa but contains some Asian species. *Lactifluus* sect. *Albati* is mainly temperate with representatives from North America and Europe, but some Asian species are also placed in this group. The molecular analyses contradict the monophyly of *L.* subgenus *Lactariopsis*. *Lactifluus* sect. *Albati* is an evolutionarily distinct group that is not closely related to *L.* sect. *Lactariopsis* and *L.* sect. *Chamaeleontini*. The monophyly of both latter sections remains to be further investigated. We provisionally maintain the traditional division of *L.* subg. *Lactariopsis* in three sections here for practical reasons.

Lactifluus subg. ***Lactariopsis*** (Henn.) Verbeken, **comb. nov.**
MycoBank MB 563214
≡ *Lactariopsis* Henn., Bot. Jahrb. Syst. 30: 51. 1901.
≡ *Lactarius* sect. *Lactariopsis* (Henn.) Singer, Ann. Mycol 40: 111. 1942 (as "*Lactariopsideae*").
≡ *Lactarius* subg. *Lactariopsis* (Henn.) R. Heim, Prodr. Fl. Mycologique Madagascar 1: 36. 1938.
Type species: *Lactariopsis zenkeri* Henn.

Lactifluus sect. ***Lactariopsis*** Verbeken, **sect. nov.**
MycoBank MB 563215
Velum praesens distinctumque, annulum formans. Pileipellis et stipitipellis cum pilis crassiparietini abundantes. Pseudopleurocystidia abundantia, fusiformia, emergentia.
Type species: *Lactariopsis zenkeri* Henn.

Lactifluus annulatoangustifolius (Beeli) Buyck, **comb. nov.**
MycoBank MB 563170
≡ *Russula annulatoangustifolia* Beeli, Bull. Jard. Bot. État 14: 87. 1936.
≡ *Lactarius annulatoangustifolius* (Beeli) Buyck, Bull. Jard. Bot. Belg. 59: 241. 1989.

Lactifluus annulifer (Singer) Nuytinck, **comb. nov.**
MycoBank MB 563171
≡ *Lactarius annulifer* Singer, Beih. Nova Hedwigia 77: 290. 1983.

Lactifluus heimii (Verbeken) Verbeken, **comb. nov.**
MycoBank MB 563172
≡ *Lactarius heimii* Verbeken, Bull. Jard. Bot. Belg. 65: 201. 1996.

Lactifluus neotropicus (Singer) Nuytinck, **comb. nov.**
MycoBank MB 563173
≡ *Lactarius neotropicus* Singer, Kew Bull. 7: 299. 1952.

Lactifluus pelliculatus (Beeli) Buyck, **comb. nov.**
MycoBank MB 563174
≡ *Armillaria pelliculata* Beeli, Bull. Soc. Roy. Bot. Belgique 59: 111. 1927.
≡ *Lactarius pelliculatus* (Beeli) Buyck, Bull. Jard. Bot. Belg. 59: 142. 1989.

Lactifluus velutissimus (Verbeken) Verbeken, **comb. nov.**
MycoBank MB 563175
≡ *Lactarius velutissimus* Verbeken, Bull. Jard. Bot. Belg. 65: 212. 1996.

Lactifluus zenkeri (Henn.) Verbeken, **comb. nov.**
MycoBank MB 563176
≡ *Lactariopsis zenkeri* Henn., Bot. Jahrb. Syst. 30: 51. 1902 ("1901").
≡ *Lactarius zenkeri* (Henn.) Singer, Ann. Mycol. 40: 111. 1942.

Lactifluus sect. ***Chamaeleontini*** (Verbeken) Verbeken, **comb. nov.**
MycoBank MB 563216
≡ *Lactarius* section *Chamaeleontini* Verbeken, Mycotaxon 66: 393. 1998.
Type species: *Lactarius chamaeleontinus* R. Heim

Lactifluus chamaeleontinus (R. Heim) Verbeken, **comb. nov.**
MycoBank MB 563217
≡ *Lactarius chamaeleontinus* R. Heim, Bull. Jard. Bot. État 25: 87. 1955.

Lactifluus emergens (Verbeken) Verbeken, **comb. nov.**
MycoBank MB 563218
≡ *Lactarius emergens* Verbeken, Syst. Geogr. Pl. 70(1): 192. 2000.

Lactifluus indusiatus (Verbeken) Verbeken, **comb. nov.**
MycoBank MB 563219
≡ *Lactarius indusiatus* Verbeken, Bull. Jard. Bot. Belg. 65: 201. 1996.

Lactifluus laevigatus (Verbeken) Verbeken, **comb. nov.**
MycoBank MB 563220
≡ *Lactarius laevigatus* Verbeken, Bull. Jard. Bot. Belg. 65: 203. 1996.

Lactifluus leoninus (Verbeken & E. Horak) Verbeken, **comb. nov.**
MycoBank MB 563221
≡ *Lactarius leoninus* Verbeken & E. Horak, Austr. Syst. Bot. 12: 775. 1999.

Lactifluus madagascariensis (Verbeken & Buyck) Buyck, **comb. nov.**
MycoBank MB 563222
≡ *Lactarius madagascariensis* Verbeken & Buyck, Mycol. Res. 111: 791. 2007.

Lactifluus pruinatus (Verbeken & Buyck) Verbeken, **comb. nov.**
MycoBank MB 563223
≡ *Lactarius pruinatus* Verbeken & Buyck, Mycotaxon 66: 399. 1998.

Lactifluus sesemotani (Beeli) Buyck, **comb. nov.**
MycoBank MB 563224
≡ *Russula sesemotani* Beeli, Bull. Soc. Roy. Bot. Belgique 60(2): 169. 1928.
≡ *Lactarius sesemotani* (Beeli) Buyck, Bull. Jard. Bot. Belg. 59: 241. 1989.

Lactifluus sect. ***Albati*** (Bataille) Verbeken, **comb. nov.**
MycoBank MB 563735
≡ *Lactarius* [unranked] *Albati* Bataille, Fl. Monogr. Astéro.: 35. 1908.
≡ *Lactarius* subsect. *Albati* (Bataille) Konrad, Bull. Soc. Mycol. France 51: 188. 1935.
≡ *Lactarius* sect. *Albati* (Bataille) Singer, Ann. Mycol 40: 109. 1942.
Type species: *Agaricus vellereus* Fr. : Fr.

Lactifluus bertillonii (Neuhoff ex Z. Schaef.) Verbeken, **comb. nov.**
MycoBank MB 563226
≡ *Lactarius vellereus* var. *bertillonii* Neuhoff ex Z. Schaef., Česká Mykol. 33: 9. 1979.
≡ *Lactarius bertillonii* (Neuhoff ex Z. Schaef.) Bon, Doc. Mycol. 10(37–38): 92. 1980 ("1979").

Lactifluus deceptivus (Peck) Kuntze, Revis. Gen. Pl. 2: 856. 1891.
≡ *Lactarius deceptivus* Peck, Annual Rep. New York State Mus. 38: 125. 1885.

Lactifluus pilosus (Verbeken, H.T. Le & Lumyong) Verbeken, **comb. nov.**
MycoBank MB 563227
≡ *Lactarius pilosus* Verbeken, H.T. Le & Lumyong, Mycotaxon 102: 287. 2007.

Lactifluus puberulus (H.A. Wen & J.Z. Ying) Nuytinck, **comb. nov.**
MycoBank MB 563228
≡ *Lactarius puberulus* H.A. Wen & J.Z. Ying, Mycosystema 24(2): 155. 2005.

Lactifluus subvellereus (Peck) Nuytinck, **comb. nov.**
MycoBank MB 563229
≡ *Lactarius subvellereus* Peck, Bull. Torrey Bot. Club 25: 369. 1898.

Lactifluus vellereus (Fr. : Fr.) Kuntze, Revis. Gen. Pl. 2: 857. 1891.
≡ *Agaricus vellereus* Fr. : Fr., Syst. Mycol. 1: 76. 1821.
≡ *Lactarius vellereus* (Fr. : Fr.) Fr., Epicr. Syst. Mycol.: 340. 1838.

Lactifluus subg. *Russulopsis*

Currently, this group comprises eight species, all endemic for tropical Africa. All species are recombined in the genus *Lactifluus* here.

Lactifluus subg. ***Russulopsis*** (Verbeken) Verbeken, **comb. nov.**
MycoBank MB 563736
≡ *Lactarius* subg. *Russulopsis* Verbeken, Mycotaxon 77: 439. 2001.
Type species: *Lactarius ruvubuensis* Verbeken

Lactifluus sect. ***Russulopsidei*** (Verbeken) Verbeken, **comb. nov.**
MycoBank MB 563737
≡ *Lactarius* sect. *Russulopsidei* Verbeken, Mycotaxon 77: 440. 2001.
Type species: *Lactarius ruvubuensis* Verbeken

Lactifluus brachystegiae (Verbeken & C. Sharp) Verbeken, **comb. nov.**
MycoBank MB 563230
≡ *Lactarius brachystegiae* Verbeken & C. Sharp, Syst. Geogr. Pl. 70(1): 187. 2000.

Lactifluus claricolor (R. Heim) Verbeken, **comb. nov.**
MycoBank MB 563231
≡ *Lactarius claricolor* R. Heim, Candollea 7: 381. 1938.

Lactifluus corbula (R. Heim & Gooss.-Font.) Verbeken, **comb. nov.**
MycoBank MB 563232
≡ *Lactarius corbula* R. Heim & Gooss.-Font., Bull. Jard. Bot. État 25: 64. 1955.

Lactifluus cyanovirescens (Verbeken) Verbeken, **comb. nov.**
MycoBank MB 563233
≡ *Lactarius cyanovirescens* Verbeken, Bull. Jard. Bot. Belg. 65: 199. 1996.

Lactifluus longipes (Verbeken) Verbeken, **comb. nov.**
MycoBank MB 563234
≡ *Lactarius longipes* Verbeken, Bull. Jard. Bot. Belg. 65: 203. 1996.

Lactifluus pseudotorminosus (R. Heim) Verbeken, **comb. nov.**
MycoBank MB 563235
≡ *Lactarius pseudotorminosus* R. Heim, Candollea 7: 379. 1938.

Lactifluus ruvubuensis (Verbeken) Verbeken, **comb. nov.**
MycoBank MB 563236
≡ *Lactarius ruvubuensis* Verbeken, Bull. Jard. Bot. Belg. 65: 208. 1996.

Lactifluus urens (Verbeken) Verbeken, **comb. nov.**
MycoBank MB 563237
≡ *Lactarius urens* Verbeken, Bull. Jard. Bot. Belg. 65: 211. 1996.

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Validation of combinations with basionyms published by Fries in 1861

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Abstract — Authors (including some of us) have incorrectly cited as basionyms names treated by Fries in 1863 that were actually originally published by him in 1861. As these basionym citation errors mean that the intended new combinations are not validly published, the following combinations are again proposed as new: *Chromosera cyanophylla*, *Mythicomyces corneipes*, *Tephrocybe misera*, *T. tesquorum*. Three other intended combinations are noted as also not validly published, but the species are currently treated under the different (and validly published) names *Haasiella venustissima, Phaeoclavulina curta,* and *Rhodonia placenta.*

Key words — *Ceriporiopsis, Gerronema, Ramaria,* International Code of Botanical Nomenclature

Introduction

Fries (1861) published a series of observations on new or little known hymenomycetes from Sweden. Most of his new taxa were included again under the same names in his Monographia Hymenomycetum Sueciae (Fries 1863). In his subsequently published Hymenomycetes Europaei (Fries 1874), Fries referred back only to the 1863 publication for each of the names originally published in 1861. This has led to a series of citation errors by later authors and indices, some of which affect the valid publication of proposed combinations.

In 1863, Fries did refer back to the 1861 publication as the source for each of the names corrected below. Therefore the authors, who proposed the new combinations after 1 January 1953 and who cited only the 1863 or later publications, violated Articles 33.4 and 33.7 of the International Code of Botanical Nomenclature (McNeill et al. 2006) and did not validly publish their new combinations.

Under Art. 33.5 & 33.7(a), such citation errors are not correctable. Here we validate the previously proposed binomials that are now generally accepted and in current use, despite their current lack of status under the Code (McNeill et al. 2006). With one exception in *Haasiella*, we are unaware of incidental subsequent validations.

Chromosera cyanophylla (Fr.) Redhead, Ammirati & Norvell, **comb. nov.**

MycoBank MB 563787

≡ *Agaricus cyanophyllus* Fr., Öfvers. K. Svensk. Vetensk.-Akad. Förhandl. 18(1): 23 (1861).

Redhead et al. (1995) proposed the genus *Chromosera* Redhead, Ammirati & Norvell, citing as type "*Chromosera cyanophylla*" and listing Fries (1863) (and not the earlier Fries 1861) for the basionym. They did, however, list as obligate synonyms three validly published names: *Agaricus cyanophyllus* Fr. (albeit with the incorrect citation), *Omphalia cyanophylla* (Fr.) Quél. (Quélet 1872: 99), and *Omphalina cyanophylla* (Fr.) Quél. (Quélet 1886: 45). This indication of the type fulfilled the requirements for valid publication of the generic name (Art. 37.2), but the incorrect citation did not meet the requirements for valid publication of the binomial. Notably, the requirements for valid publication of new combinations prior to 1953 were far more lenient.

Mythicomyces corneipes (Fr.) Redhead & A.H. Sm. **comb. nov.**

MycoBank MB 563788

≡ *Agaricus corneipes* Fr., Öfvers. K. Svensk. Vetensk.-Akad. Förhandl. 18(1): 25 (1861).

Similarly, Redhead & Smith (1986) proposed the genus *Mythicomyces* Redhead & A.H. Sm., citing as type "*Mythicomyces corneipes* (Fries) comb. nov." They listed as obligate synonyms two validly published names: the first, *Agaricus corneipes* Fr., incorrectly cited Fries (1863), and the second was *Psilocybe corneipes* (Fr.) P. Karst. (Karsten 1879: 504). These actions fulfilled the requirements for valid publication of the generic name (Art. 37.2) but failed to meet requirements for valid publication of the binomial, and so we publish it here.

Tephrocybe misera (Fr.) M.M. Moser ex Contu & Vizzini **comb. nov.**

MycoBank MB 563789

≡ *Agaricus miser* Fr., Öfvers. K. Svensk. Vetensk.-Akad. Förhandl. 18(1): 21 (1861).

Tephrocybe tesquorum (Fr.) M.M. Moser ex Contu & Vizzini, **comb. nov.**
MycoBank MB 563790
≡ *Agaricus tesquorum* Fr., Öfvers. K. Svensk. Vetensk.-Akad. Förhandl. 18(1): 22 (1861).

Moser (1967) proposed two combinations in *Tephrocybe* but cited Fries (1863) rather than (1861).

Notes on other cases of names not being validly published

Haasiella venustissima (Fr.) Kotl. & Pouzar ex Chiaffi & Surault, Bull. Soc. Mycol. Fr. 112: 127 (1996).
≡ *Agaricus venustissimus* Fr., Öfvers. K. Svensk. Vetensk.-Akad. Förhandl. 18(1): 21 (1861).

Kotlaba & Pouzar (1966) erected the genus *Haasiella* Kotl. & Pouzar, typified by *H. splendidissima* Kotl. & Pouzar, and included a second species, for which they proposed the combination "*Haasiella venustissimus*," citing Fries (1863) for the basionym. Their combination was inadvertently validated by Chiaffi & Surault (1996), who cited the basionym and correct place of publication. The proposed combination "*Gerronema venustissimum*" by Singer (1962) is not validly published, as only Fries (1863) was cited.

Phaeoclavulina curta (Fr.) Giachini, Mycotaxon 115: 190 (2011).
≡ *Clavaria curta* Fr., Öfvers. K. Svensk. Vetensk.-Akad. Förhandl. 18(1): 31 (1861).

The intended combination in *Ramaria* by Schild (1994) was not validly published because he referred only to Fries (1863) "Monogr. Hym. Suec.," which he mistakenly cited as "1857," the date of the earlier parts of that title. Giachini & Castellano (2011) correctly cited Fries (1861).

Rhodonia placenta (Fr.) Niemelä, K.H. Larss. & Schigel, Karstenia 45(2): 79 (2005).
≡ *Polyporus placenta* Fr., Öfvers. K. Svensk. Vetensk.-Akad. Förhandl. 18(1): 30 (1861).

Domański's (1963) intended combination in *Ceriporiopsis* Domański, where he cites Fries (1874), was not validly published. Niemelä et al. (2005) correctly cited Fries (1861).

Acknowledgements

We thank Shaun Pennycook and John McNeill for reviewing the manuscript and confirming that the genus *Chromosera* was validly published according to the ICBN even though the earlier combination of *Chromosera cyanophylla* was not validly published.

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Book reviews and notices

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Introduction

Three totally different books are reviewed here: a beautifully illustrated French book on myxomycetes, an Indian book on the smut fungi of the subcontinent, and a well-written, popular-science introduction to mushroom forming fungi, succinctly titled 'Mushroom.'

Book announcements include the 2011 publications in the series 'Fungi non delineati,' a very nicely illustrated book on tropical fungi in China, and a checklist for all fungi and myxomycetes of Rhode Island (U.S.A.).

Myxomycetes

Les myxomycètes. By M. Poulain, M. Meyer & J. Bozonnet, 2010. FMBDS, 8, Avenue de la Plaine, 74000 Annecy, France, <philippecattin74@orange.fr>. 2 vols, pp. 1119, col. plates 544. ISBN 978-2-9518540-2-4. Price 159.00 €

As anyone who has ever studied myxomycetes knows, the monograph by Martin and Alexopoulos, published by the University of Iowa Press in 1969, represents the single most definitive treatment of this group of organisms published to date. This monograph (The Myxomycetes) has long since been out of print, but it still remains—more than 40 years after it first appeared—the standard against which any of the more recently published works on the myxomycetes are judged. The two volumes that make up Les Myxomycètes undoubtedly represent the most noteworthy recent contribution to the study of myxomycetes. Although not totally comprehensive, since some species (particularly tropical examples) are not included, this work covers virtually any species one is likely to encounter

* Books for consideration for coverage in this column should be mailed to the Book Review Editor at the address above. All unsigned entries are by the Book Review Editor.

during a lifetime of collecting. Information is provided on 853 taxa (species and varieties), and 530 of these are illustrated with color images. These images represent the most important feature of the work itself. Never before have so many high-quality (often stunning) macroscopic color images been provided for so many different myxomycetes. For anyone studying myxomycetes, this alone is reason enough to obtain a copy of Les Myxomycètes. The first of the two volumes begins with a short introduction to the biology, ecology and morphology of myxomycetes. Since the primary language used throughout the work is French, this information is not readily available to everyone. However, the remainder of the first volume is devoted to keys and short descriptions of the species being considered, and this material is presented in both French and English, which is a major plus for those of us whose knowledge of the former language is limited. The descriptions include both macroscopic as well as microscopic characters, and reference is made to the number of the image provided for a particular species in the second volume. The first volume also contains a glossary of terms, an index to all of the taxa being considered and a list of relevant bibliographical references. The nomenclature used throughout both volumes is that found in the current version of Nomenmyx (www.nomen.eumycetozoa.com), which is generally followed by most of us who work with myxomycetes.

The second volume contains the color images of the 530 taxa. The general approach used throughout is to have each page devoted to a single image that shows the habit of the species in question, but some pages contain several images of the same species. Also included are line drawings that show various microscopic structures (e.g., spores, capillitium and peridium) that are relevant to that species. Altogether, the images, drawings and descriptions provide enough information to allow most specimens of myxomycetes to be identified with a high degree of certainty. Since the authors are especially well known for their studies of nivicolous ("snowbank") myxomycetes, there is extensive coverage of this group. In fact, information on some of the nivicolous taxa they have included in this work was available previously only in the primary literature. The majority of the color images are truly outstanding, and the only complaint that anyone might have is that in a few instances (e.g., *Physarum pusillum*) the specimen photographed might not have been absolutely typical for a given species. I found the images of the various species of *Licea* particularly well done and thus potentially very useful when working with members of this genus.

Overall, Les Myxomycètes is an extraordinary work that is the result of an enormous amount of time and effort on the part of the three authors. Simply looking through the color images in the second volume is enjoyable, but the complementary information provided in the first volume makes this an

exceedingly useful scientific work on the myxomycetes. Anyone with even the slightest interest in this group of organisms should have a copy of this work.

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Ustilaginales

***Ustilaginales* of India.** By R.V. Gandhe, 2011. Bishen Singh Mahendra Pal Singh, 23-A, New Connaught Place, Dehra Dun, 248001 India, <bsmps@vsnl.com>. ISBN 978-81-211-0788-4. Pp. 414, col. pl. 22, figs. Price circa US$ 154

Dedicated to 'the doyen of mycology in India' the late Professor M.J. Thirumalachar, this book gives an overview of the *Ustilaginales* (s.l.) species in India.

The economically important smut fungi of crop plants and the smut fungi of native plants are treated in this book. It gives descriptions of 343 species in 45 genera, several of them new for science (e.g., three new *Sorosporium* species and two new *Sphacelotheca* species), the genera and species per genus are treated in alphabetical order. The genus *Sporisorium* is the largest, represented by 96 species, followed by *Tilletia* with 46, and *Ustilago* with 42.

Besides an introduction to smut fungi and coverage of their economic importance, diversity, and morphological variations, a useful host index is provided.

The author, who has spent 2 decades studying smut fungi and especially their germination patterns, places a strong emphasis on morphotaxonomy and symptomatology, and although in many cases descriptions are literally the same as in Vánky (2007), morphological characters have been added to the keys. The illustrations entail eight colour plates and over 50 drawings of infected plants, spores, and germinating spores.

The book makes the impression of having been written at the same time as Vánky's treatment of the smut fungi of the Indian subcontinent was published, although that publication is lacking from the list of references. There are no post-2007 publications cited, not even the phylogenetic paper by Begerow et al. (2007). The illustrations in Vánky's 2007 work are of higher quality than the ones in the present book, but I doubt whether the former is easily available in India, where it should be used.

Begerow D, Stoll M, Bauer R. 2007 ['2006'] A phylogenetic hypothesis of *Ustilaginomycotina* based on multiple gene analyses and morphological data. Mycologia 98: 906–916.

Vánky, K. 2007. Smut fungi of the Indian Subcontinent. Polish Botanical Studies 26.

MISCELLANEOUS

Mushroom. By N.P. Money, 2011. Oxford University Press, 198 Madison Avenue, New York, NY 10016, U.S.A. <www.oup.com>. ISBN 978-0-19-973256-2. Pp. 201, col. pl. 16, figs. Price US$ 24.95

In eight chapters the reader is given a dazzling, fast-spaced and thorough introduction into the development of mushrooms (including life cycles), spore launching, diversity in shape and function, edible mushrooms and conservation, mushroom cultivation, toxicity, intoxication, and the medicinal mushroom industry. The text moves back and forth between the author's experiences in the England of his youth and present-day life, science, and economics and yet is always anchored safely in the writings and illustrations of the scientists of the past. Central to all is the biology of the mushroom, as masterpiece of bioengineering. It is by no means a text book or field guide. Nonetheless, MUSHROOM gives a huge amount of information in its 200 pages, makes for excellent reading, and would be a good present to friends, family, and all who are intrigued by nature.

BOOK ANNOUNCEMENTS

Characteristic and rare species of gasteromycetes in Eupannonicum. By I. Rimóczo. M. Jeppson & L. Benedek, 2011. FUNGI NON DELINEATI 56-57. Edizioni Candusso, Via Ottone Primo 90, 17021 Alassio SV, Italy. <maxcandusso@libero.it>. Pp. 230, col. plates 108, figs. Price 28.00 €

***Cortinarius* Ibero-insulares – 3.** By Grupo Iberoinsular de Cortinariologos (GIC), 2011. FUNGI NON DELINEATI 58-59. Edizioni Candusso, Via Ottone Primo 90, 17021 Alassio SV, Italy. <maxcandusso@libero.it>. Pp. 236, col. plates 236. Price 33.00 €

Fungi of tropical China. By W. Xingliang et al., 2011. Chinese Corporation for Promotion of Humanities. ISBN-13: 9787030294708. Pp. 548, col. plates 495. Price circa US$ 220

***Inocybe* dai litorali alla zona alpina.** By E. Ferrari, 2011. FUNGI NON DELINEATI 54-55. Edizioni Candusso, Via Ottone Primo 90, 17021 Alassio SV, Italy. <maxcandusso@libero.it>. Pp. 216, col. plates 106, figs 69. Price 23.00 €

Mycota of Rhode Island: A checklist of the fungi recorded in Rhode Island (including lichens and myxomycetes). By R.D. Goos, 2010. THE BIOTA OF RHODE ISLAND vol. 4. Rhode Island Natural History Survey, P.O. Box 1858, Kingston, RI 02881, <programadmin@rinhs.org>. ISBN 1-887771-09-3. Pp. 228. Price US$60.00

Rare and noteworthy species of agarics from the western Caucasus. By E.F. Malysheva & T. Yu. Svetasheva, 2011. FUNGI NON DELINEATI 60. Edizioni Candusso, Via Ottone Primo 90, 17021 Alassio SV, Italy. <maxcandusso@libero.it>. Pp. 104, col. plates 45. Price 12.00 €

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bad taxonomy

can KILL